Risk factors for cognitive decline in older people with type 2 diabetes

Volume I:

Introduction, Method and Results I

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Declaration

I, Insa Feinkohl, declare that this thesis is my own composition. The work presented here has not been submitted for any other degree or professional qualification.

The Edinburgh Type 2 Diabetes Study, which provided the data for all analyses presented here, had already completed the baseline and year 1 clinics by the time I joined the study, but I was involved in the collection of cognitive data at the year 4 follow-up clinic in 2010/2011. Therefore, all of the baseline clinical variables and the baseline cognitive test data used for the purpose of the analyses presented in this thesis were collected, cleaned and (in some cases) manipulated through the efforts of other members of the research team. However, all of the statistical analyses on the basis of this data were performed by myself.

Some of the findings from analyses presented in this thesis were published in scientific journals. The manuscripts are included in the Appendix with permission from Diabetes Care®.

Signed: ________________________

Date: 05/05/2014

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Abbreviations

ABI	ankle brachial pressure index
BVFT	Borkowski Verbal Fluency Test
CI	confidence interval
cIMT	carotid intima-media thickness
CRP	C-reactive protein
CVD	cardiovascular disease
DR	diabetic retinopathy
DSC	Digit Symbol Coding
Faces	Faces test
HADS	Hospital Anxiety and Depression Scale
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	interleukin-6
LM	Logical Memory
LNS	Letter-Number Sequencing
MI	myocardial infarction
MMSE	Mini-Mental-State Examination
MR	Matrix Reasoning
MVD	macrovascular disease
NART	National Adult Reading Test
NT-proBNP	N-terminal pro-brain natriuretic peptide
OR	odds ratio
PAD	peripheral arterial disease
RCT	randomised controlled trial
SD	standard deviation
SES	socioeconomic status
SH	severe hypoglycaemia
St. Error	standard error
TIA	transient ischaemic attack
TMT-B	Trail-Making Test-B
TNF-α	tumor necrosis factor-α
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale

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Abstract

People with type 2 diabetes are at increased risk of age-related cognitive impairment. Previous literature has focused on case-control studies comparing rates of cognitive impairment in patients with and without diabetes. Investigations of potential risk factors for cognitive impairment (including those with increased prevalence in diabetes, such as macrovascular disease, and diabetes-specific factors such as hypoglycaemia) in study populations consisting exclusively of patients with type 2 diabetes have been largely neglected. Moreover, previous studies have failed to take advantage of the extensive characterisation and prospective nature of longitudinal cohort studies to investigate the relative predictive ability of a wider range of potential risk factors for cognitive decline. Using data from the prospective Edinburgh Type 2 Diabetes Study (ET2DS) the present thesis aimed (i) to determine associations of cognitive decline with macrovascular disease and with severe hypoglycaemia, and (ii) to compare a wider range of potential risk factors in their ability to predict cognitive decline.

In 2006/2007, 1066 patients with type 2 diabetes (aged 60 to 75 years) attended the baseline ET2DS clinic and 831 returned for the follow-up at year 4. Subjects were extensively characterised for risk factor profiles at baseline, and at year 4 for incidence of severe hypoglycaemia. Socioeconomic status was estimated using postcode data. Scores on seven tests of age-sensitive ‘fluid’ cognitive function, which were administered at baseline and at year 4, were used to derive a general cognitive component (*‘g’*). A vocabulary-based test, administered at baseline, estimated pre-morbid ability. Findings are reported in three parts. 1.) *Macrovascular disease and cognition*: Subjects with higher levels of biomarkers indicative of subclinical macrovascular disease, including plasma N-terminal pro-brain natriuretic peptide and carotid intima-media thickness, had significantly steeper four-year cognitive decline, independent of traditional cardiovascular risk factors, stroke, socioeconomic status and estimated pre-morbid cognitive ability. For ankle-brachial pressure index, the association fell just short of statistical significance. Effect sizes were overall modest, with fully adjusted standardised beta coefficients ranging from 0.06 to -0.12. Little evidence was found for associations of the symptomatic markers of macrovascular disease with four-year change in cognitive function that was

independent of participants' pre-morbid ability and socioeconomic status. 2.) *Severe hypoglycaemia and cognition*: Subjects with lower cognitive ability at baseline were at two-fold increased risk of experiencing their first-ever incident severe hypoglycaemia during follow-up. The rate of four-year cognitive decline was significantly steeper in those exposed to hypoglycaemia compared with hypoglycaemia-free participants, independently of cardiovascular risk factors, micro- and macrovascular disease and of estimated pre-morbid cognitive ability. Effect sizes again were overall modest (Cohen's d = 0.2 to 0.3 for statistically significant differences in four-year cognitive decline between subjects with and those without hypoglycaemia, following multivariable adjustment) 3.) *Consideration of a wider range of risk factors and cognition*: A stepwise linear regression model including a total of 15 metabolic and vascular risk factors identified inflammation, smoking and poorer glycaemic control (in addition to some of the subclinical markers of macrovascular disease) as predictive of a steeper four-year cognitive decline. Other traditional cardiovascular risk factors, diabetic retinopathy, clinical macrovascular disease and a baseline history of severe hypoglycaemia were not included in this model. The interpretation of the latter finding is limited, however, by the fact that the stepwise regression procedure may exclude true predictors from a model when they correlate with already included risk factors.

This thesis has demonstrated associations of later-life cognitive decline in people with type 2 diabetes with markers of subclinical macrovascular disease and poor glycaemic control (including hypoglycaemia) as well as other cardiometabolic risk factors (inflammation, smoking). Findings suggest that associations are relatively weak and complex due to inter-relationships amongst risk factors, and indicate a role of pre-morbid ability and socioeconomic status (which as risk factors are difficult to modify) in the relationships of risk factors with cognitive decline. Future research including case-control studies to compare risk factor associations between people with type 2 diabetes and non-diabetic older adults and randomised controlled trials to evaluate potential causal effects of individual modifiable risk factors on cognitive decline, will help to evaluate the mechanisms underlying the observation that people with type 2 diabetes are at risk of cognitive impairment in later life.

Chapter 1: Introduction: Type 2 diabetes mellitus and cognition

1.1 Diabetes Mellitus

Glucose is the 'fuel' required for the body to function but becomes available as energy to its cells only through an interaction with the hormone insulin. When the glucose metabolism of a person is functioning correctly, insulin levels reflect the glucose levels present in the blood. In diabetes mellitus, or diabetes, this delicate balance is lost.

1.1.1 Pathology and diagnosis

In healthy glucose metabolism, preproinsulin is secreted from the β-cells of the pancreas and is converted to insulin when blood glucose levels rise. Insulin then stimulates glucose transport into muscle and adipose cells and reduces glucose production in the liver, overall reducing the glucose levels present in the blood (Rains & Jain, 2011). Diabetes is characterised by chronically raised blood glucose levels (hyperglycaemia) and mainly occurs as type 1, type 2 and a gestational form. It may also develop in response to medication (iatrogenic diabetes), due to genetic defects or diseases of the pancreas (ADA, 2011). Ninety to 95% of newly diagnosed cases are of type 2 (Centers for Disease Control and Prevention, 2011). This form of diabetes, also referred to as non-insulin dependent diabetes mellitus (NIDDM), is the focus of this thesis and 'diabetes' implies type 2 diabetes unless otherwise specified. Compared with patients with other types of diabetes, type 2 diabetes patients are typically older and are consequently more commonly affected by co-morbidities. In the development of the disease, the body gradually loses its ability to control blood glucose levels because insulin sensitivity declines due to progressive hypo-responsiveness of the insulin receptors (IR) (Zhao & Townsend, 2009). Initially, this is compensated for by an increase in insulin secretion, but subsequent reduction in β-cell function ultimately results in hyperglycaemia (Biessels, 2010). Common risk factors for type 2 diabetes include a family history of diabetes, South Asian or black ethnicity and a prior history of gestational diabetes, as well as obesity, a hypercalorific diet and physical inactivity (Wild & Byrne, 2006).

The specific diagnostic criteria for diabetes vary between institutions and are not undisputed (Resnick & Harris, 1999). According to the American Diabetes Association (ADA, 2011), a single reading of fasting glucose levels above 7.0 mmol/L (no caloric intake for at least eight hours), a casual reading of above 11.1 mmol/L (without regard to time since last meal), or a reading of above 11.1 mmol/L in an oral glucose tolerance test (OGTT) during which the effect of an ingested glucose solution on fasted blood levels is tested, is sufficient for diagnosis. The World Health Organisation (WHO) accepts fasting plasma glucose levels above 7.0 mmol/l or plasma glucose above 11.1 mmol/l two hours following an OGTT (WHO, 2006). Recently, both ADA (2011) and the WHO (2011) extended their criteria to accept glycated haemoglobin levels (HbA1c), which reflect average blood glucose levels over two to three months, of above 6.5% as indicative of diabetes.

Disease onset in type 2 diabetes is gradual, and several pre-diabetic states have been described. Impaired glucose tolerance (IGT) occurs when fasting plasma glucose levels are below the diagnostic criteria for diabetes (<7 mmol/l), but two-hour post-OGTT results are 7.8 mmol/l to 11.1mmol/l. Impaired fasting glucose (IFG) is defined as fasting glucose levels between 6.1 and 6.9 mmol/l, with two- hour post-OGTT below 7.8 mmol/l if measured (WHO, 2006). Around one third of individuals with IGT or IFG remain pre-diabetic, one third revert to normoglycaemia, and one third progress to diabetes (Garber, 2012). A large proportion of people with diabetes remain undiagnosed because symptoms are relatively mild. Patients may experience excessive thirst, increased urination, weight loss, blurred vision, impairment of growth and susceptibility to certain infections (ADA, 2002). Diabetes has serious long-term complications, including retinopathy, chronic renal disease, autonomic and peripheral neuropathy, macrovascular disease (including coronary artery disease and stroke), sexual dysfunction and urinary incontinence (ADA, 2002; Centers for Disease Control and Prevention, 2011). Overall, quality of life is reduced and activities of daily living are compromised (Bruce et al., 2003; Rubin & Peyrot, 1999).

1.1.2 Global Prevalence

Diabetes is not a modern day occurrence - the condition was already alluded to by Hippocrates and Aristotle in ancient Greece. In the first century AD, diabetes was described in some detail by Aretaeus as “melting down of the flesh into urine” (Gemmill, 1972). Today, diabetes is a global health concern, following a rapid increase in prevalence which has resulted in a diabetes ‘epidemic’ (Olhansky et al., 2005). Whereas in 1935, 15 million people worldwide were estimated to be suffering from a form of diabetes (1% of the world population of 2 billion), this number exploded to 220 million in 2010 (over 3% of a world population approaching 7 billion) (Cahn & O'Brien, 1996; US Census Bureau, 2000b). Changes in diagnostic criteria since 1965 (WHO, 2006) and improved case ascertainment partly account for this development. However, actual increases in fasting plasma glucose levels in the Western world (Danaei et al., 2011) are undeniable. In the US, which some have labelled the ‘United States of Diabetes’, and which already sees any form of diabetes as the seventh most common cause of death (Centers for Disease Control and Prevention, 2011), prevalence of any type of diabetes is expected to rise from 12% in 2010 to 15% by 2020 (United Health Center for Health Reform & Modernization, 2010). In Scotland, estimated prevalence was 9% in 2008, following a rise from 5% in 2003 (Hamer, Kengne, Batty, Cooke, & Stamatakis, 2011). This development can be mainly attributed to an increasingly sedentary lifestyle and excessive consumption (F. B. Hu, Li, Colditz, Willett, & Manson, 2003), which has led to a decrease in the average age at diagnosis (Harron et al., 2011; Launer, 2009). At the same time, society is ageing rapidly. In the US, the proportion of over 65s has increased from 4% to 12% since 1900 (US Census Bureau, 2000a, 2004, 2011; Warsch & Wright, 2010) and life expectancy may reach 100 within the current century (Oeppen & Vaupel, 2002).

1.2 Intelligence and g

1.2.1 Introduction and terminology

Because diabetes has been associated with intellectual deficits and this thesis aims to identify some of the underlying mechanisms, the concept of intelligence and its relationship with ageing is briefly introduced in the sections to follow.

Intelligence is usually defined as the ability to learn, understand and deal with novel situations (W. Johnson & Bouchard, 2005), and is consequently required for automated as well as for higher order mental tasks. Individual differences in intelligence test scores are associated with occupational achievements, social mobility and longevity (Deary, Penke, & Johnson, 2010). It is understood that the genetic make-up of an individual strongly contributes to their performance on intelligence tests. Over 50% of variance in test scores at age 65 may be accounted for by genetic factors, for instance. Yet, the precise 'locus' of intelligence is unclear. Although researchers have attempted to identify potential distinct brain areas or networks responsible, the evidence overall suggests a higher general efficiency in the brains of individuals with higher compared with those with lower intelligence (Deary, 2012).

The term intelligence, which today is often avoided (Deary, 2000), is sometimes used interchangeably with cognitive ability, cognitive function, mental ability or IQ. Interest in the quantification of cognitive ability arose in the late 19^{th} century. For instance, Galton (1869; cited in (Gould, 1981)) attempted to determine individual differences in and heredity of intelligence through anthropometry, including craniometry. Whereas physiological measurements to an extent indeed reflect individual differences in cognitive ability (Deary, 2012), this kind of research is vulnerable to subjective interpretation as well as the misuse for the justification of ethnic inferiority. Consequently, the beginning of the 20^{th} century saw the development of more objective methods, which involved psychometric testing on intelligence scales similar to those still used today (Binet, 1908; cited in (Gould, 1981)).

1.2.2 Hierarchical structure of intelligence

Spearman's 2-factor theory of intelligence

Scores on scales of cognitive abilities are typically normally distributed, with slightly wider distributions for males than for females (Deary, et al., 2010). Moreover, individuals who perform well on one test tend to also perform well on another. In his '2-factor theory of intelligence', Spearman (1904) identified this pattern, applied factor analysis to scores on a number of individual tests, and named the resulting shared factor '*g*' - a measure of global cognitive ability. The use of psychometric scales of cognitive ability simply necessitated the presence and calculation of *g*, according to Spearman (Gould, 1981). As part of his theory, he further specified that each individual test resulted in an additional factor unique to that test. Consequently, individual differences in cognitive abilities would follow a hierarchical structure, with a test-specific first level, or stratum, and a global *g* stratum (Carroll, 1993). In support of Spearman's account, *g* is indeed observed in virtually all cases when more than one cognitive test is administered and accounts for around 40% of variance when 10-15 individual tests are included. The highest 'saturations' or factor loadings on *g* are typically found for tests involving spatial ability and reasoning (Deary, et al., 2010). Until relatively recently, it was believed that the nature of *g* might differ, depending on the specific tests from which it was extracted (Carroll, 1993), but repeat testing of large samples have revealed strong correlations amongst *g* derived from different standardised test batteries, demonstrating that *g* in fact arises independent of the input (W. Johnson, Bouchard, Krueger, McGue, & Gottesman, 2004; W. Johnson, te Nijenhuis, & Bouchard, 2008).

Since their discovery, Spearman's *g* and the assumed hierarchical structure of cognitive abilities have occasionally been contested despite this highly consistent evidence. In a theory which assumed a single stratum, the psychometrist Louis L. Thurstone initially suggested the presence of at least seven 'primary mental abilities' (PMA) in absence of an overarching stratum *g*, although he later acknowledged that *g* indeed became apparent in his own data sets (Carroll, 1993). The psychometrist Howard E. Gardner similarly proposed a number of intelligences in specific fields, such as 'musical intelligence', which were also all placed on a single stratum. Importantly, Gardner based his account solely on studies of gifted individuals and of brain damage. He did not apply factor analysis to his data (Carroll, 1993), which would have revealed correlations of his specific 'intelligences' with a general ability factor *g* (Deary, 2012).

Carroll's 3-stratum theory of intelligence

A majority of other theorists agreed with the evidence for a hierarchical structure of cognitive abilities, and based their own accounts on Spearman's work. For instance, relatively early on it was suggested that Spearman's 2-factor theory may be extended to include an additional 'group factor' level (Burt 1917; cited in (Carroll, 1993)). This notion was not formally conceptualised until 20 years ago, when in his '3-stratum theory' based on analyses of more than 460 individual data sets, Carroll (1993) added one stratum between the third general stratum *g* and the first stratum which (similar to Spearman's test-specific factor) is relatively narrow and reflects specialisation in abilities dependent on individual's experiences, learning opportunities and the adoption of specific strategies (W. Johnson & Bouchard, 2005). This novel intermediate stratum is based on general abilities in several broad 'cognitive domains' (W. Johnson & Bouchard, 2005), and therefore constitutes the level at which variance is shared by a number of individual cognitive tests (Deary, 2012). Despite the clear hierarchical structure, Carroll's model does not imply that higher strata dominate over lower strata. Rather, each stratum partials out the respective higher strata, so that for instance the first stratum represents a test-specific level which is not accounted for by the second and third strata (Carroll, 1993).

Carroll (1993) specified his second stratum (which accounts for relatively little variance when compared with the remaining two strata (Deary, 2012)), as the domains of memory and learning, general retrieval, visual and auditory perception, speediness, and fluid and crystallised intelligence. Herein, Carroll provided an alternative to a previous suggestion for a conceptual distinction of Spearman's *g* into the domains of 'v:ed' (verbal fluency, divergent thinking, numerical abilities, scholastic knowledge) and 'k:m' (perceptual speed, psychomotor and physical skills, spatial and mechanical abilities) brought forward by the intelligence researcher

Philip E. Vernon (1964 cited in (W. Johnson & Bouchard, 2005). Instead, Carroll followed the earlier and more commonly employed distinction of *g* into fluid intelligence (*gf*) and crystallised intelligence (*gc*), which had been suggested in the mid-20th century by Raymond Cattell and his student John L. Horn (1941 and unpublished; cited in (Cattell, 1963)).

The domain of fluid intelligence describes a wide range of abilities required to solve and adapt to novel problems (Cattell, 1963). For each, specific brain areas and networks are recruited, although these may be difficult to separate out in neuroimaging studies due to common overlaps in test demands and due to individual differences in the adoption of strategies during test performance (Brancucci, 2012).

Crystallised ability describes a person's knowledge in a specific field which is consolidated over time through the application of general ability according to personal interest (Cattell, 1963). It is influenced by personality, motivation, cultural norms and education and cannot be built up on the basis of fluid ability alone (Cattell, 1963; W. Johnson & Deary, 2011). Additionally, crystallised ability also reflects abilities which are learned more passively, such as vocabulary abilities. This is useful given that vocabulary abilities are unlikely to be affected by personal circumstances, as is the case for other estimators such as a person's knowledge in a specific field or their education. Due to this, crystallised ability (at least in English-language studies) is commonly estimated using tests in which subjects either pronounce given words of various difficulty or identify synonyms of given words (Franzen, Burgess, & Smith-Seemiller, 1997).

Fluid and crystallised abilities are highly correlated. It has been suggested that the strength of their relationship may peak in childhood, with subsequent decreases in effect sizes due to differences between individuals in the 'willingness' to invest their fluid abilities into attaining crystallised ability (W. Johnson & Bouchard, 2005). As aforementioned, Carroll (1993) positioned processing speed alongside fluid and crystallised intelligence at the second stratum of his model. Speed has been found to consistently and strongly correlate with fluid ability, and relatively weakly with tests of crystallised ability (Sheppard & Vernon, 2008). However, correlational evidence is unable to establish whether or not Carroll's positioning of speed is appropriate, or whether perhaps speed could causally influence fluid and crystallised abilities, as has since also been suggested (Deary, 2012).

Other accounts

Clearly, Carroll's specification of his second stratum is not definitive. Researchers have yet to reach agreement with regard to the number, nature and labels of cognitive domains (Deary, 2012), although at least the Cattell-Horn distinction into fluid and crystallised intelligence appears relatively well-accepted. Carroll also notes that his list of three strata is not necessarily exhaustive. Indeed, Johnson and Bouchard (2005) recently suggested and empirically supported the existence of one further intermediate level between Carroll's second and third strata. This level may be characterised by general verbal, perceptual and image rotation abilities, according to the account.

Irrespective of the precise number of strata, the literature appears to have reached consensus with regards to a hierarchical structure of cognitive abilities. All hierarchical models have in common one general factor *g* at the highest level, and this will be used as the main outcome in the analyses described in this thesis.

1.3 Cognitive ageing

1.3.1 Patterns of cognitive ageing

During ageing, we experience a global degeneration which becomes apparent in telomere shortening, mitochondrial mutations, depositions of waste products and oxidative damage, all of which equally affect the body and the brain, and their functions (Kirkwood, 2005; Liu & Mori, 1999). Crystallised abilities are relatively robust (although these may decline in advanced dementia) whereas fluid abilities are more vulnerable to age-related declines (Richards & Deary, 2005).

There is some disagreement on the precise starting point of declines in fluid abilities, with arguments brought forward for a lifelong decline with onset in the twenties, or a later onset around age 60. A majority of the evidence comes from studies with cross-sectional designs which compare cognitive performance across age groups. These studies tend to reveal early cognitive declines, with variations in trajectories and rates between the different specific abilities. Relatively automated abilities such as short-term memory may experience slower rates of decline when compared with reasoning abilities or visual and prospective memory, for instance (Hedden & Gabrieli, 2004; Logie & Maylor, 2009; Salthouse, 2009). Lifelong declines, with onset at least prior to age 60, is consistent with the observation that dementia may be preceded by two to three decades of increasing neuropathological burden (Singh-Manoux et al., 2011a).

Yet, findings from cross-sectional studies may overestimate cognitive declines due to specific recruitment methods (for instance targeting low-functioning care home populations (Hedden & Gabrieli, 2004)), as well as cohort effects. Generational differences in social and cultural environments and disease prevalence tend to favour later born cohorts in terms of cognitive function and rates of cognitive decline (although this may not extend to the declines which occur shortly before death) (W. Johnson, et al., 2004; Schaie, 2009). Further speaking against early cognitive declines, cognitive impairment is a disease of older age and even subtle cognitive deficits typically do not become apparent until later in life. Yet, advocates of 'lifelong declines' note that this may be due to compensatory processes involving increased crystallised abilities, motivation or persistence. Moreover, cognitive deficits may not become apparent earlier, because maximum cognitive performance is rarely required in everyday situations, which may additionally be modified to reduce cognitive demands (W. Johnson, et al., 2008).

Unaffected by cohort effects and other between-subject confounding, prospective evidence has particularly high validity, but findings from prospective investigations directly contrast cross-sectional findings and typically show that cognitive abilities are in fact relatively preserved or even improve until around age 60 (Salthouse, 2009; Schaie, 2004) (although the evidence is mixed, with some reports of longitudinal declines during middle-age (Singh-Manoux, et al., 2011a)). Defenders of the 'lifelong decline' contention note that practice effects and selective attrition may play a role here, however, to under-estimate the degree of cognitive ageing (Hedden & Gabrieli, 2004; Salthouse, 2009). Furthermore, longitudinal studies on cognitive ageing are typically focused on middle-age or later life, so that it is more difficult to draw conclusions on earlier declines (Tucker-Drob, 2011). Most studies are also vulnerable to random intra-individual variability, because they involve only two waves of observation (Wilson et al., 2002).

At around age 60, cross-sectional and prospective studies begin to demonstrate agreement, both providing strong evidence for cognitive decrements. Irrespective of the question of the precise ‘starting point’, it is therefore clear that fluid cognitive abilities are subject to an age-related decline. These declines appear to become sharpest after age 70 (Hedden & Gabrieli, 2004). Different mechanisms may be responsible, including increasing neuropathological burden (further described in Section 1.4) and increasing neglect of skills which engage and practice cognitive abilities (Schaie, 2004). Despite strong relationships with level of cognitive function, genetic factors do not appear to be major contributors to cognitive decline (Deary, 2012).

1.3.2 Continuum of cognitive decline and terminology

Within individuals, the rates of declines in the various cognitive abilities appear to correlate to some extent, so that people who decline in one domain tend to also decline in another (Tucker-Drob, 2011; Wilson, et al., 2002). Most importantly, large inter-individual differences are observed in the rates and trajectories of cognitive declines, and these are the subject of this thesis. Individuals whose cognitive ability is preserved or even slightly improves with age, as well as ‘normatively declining’ individuals, who see their cognitive ability compromised but do not reach the level of dementia, and patients who experience various stages of impairment and are eventually diagnosed with dementia all lie on one continuum, as is illustrated in Figure 1.1. The three groups presented in the figure are only examples; cognitive trajectories may lie anywhere in the space between the two dashed lines, which reflect complete preservation of function and pathological cognitive ageing, respectively. The distinctions between the groups, as well as the distinctions between the various clinical phases experienced by the pathological group (bottom dashed line), are all blurred.

At the individual level, the trajectories of declines are also unlikely to be captured as consistent slopes. Phases of improvements and decrements are likely. As well as downward conversion (e.g., from ‘normative decline’ to the pathological pathway leading up to dementia), long-term recovery of cognitive function (e.g., transition from the pathological pathway to ‘normative decline’) is possible up to the point of dementia diagnosis.

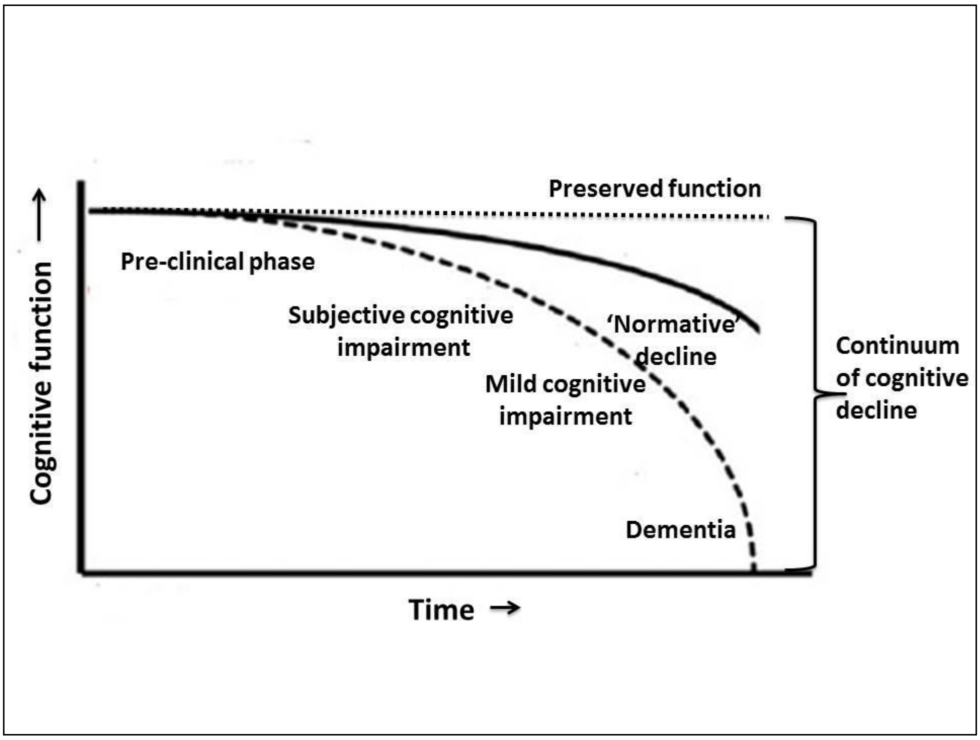

Figure 1.1: Cognitive trajectories spanning preserved cognitive function, ‘normative decline’ and the clinical course of dementia (adapted from Sperling et al., 2011)

Throughout this thesis, the terms ‘cognitive dysfunction’ as well as ‘poor’ or ‘reduced cognitive function’ all describe a lower level of cognitive ability than would be expected at that age as a consequence of ‘normative declines’. This reduction in ability may or may not reach the level of impairment and dementia. Similarly, ‘cognitive decline’ refers to a degree of decline or impairment which is accelerated relative to normative decline. When accelerated cognitive decline or impairment in the dementia-free range is referred to, this will be specified as such (‘cognitive decline short of dementia'). The latter is at the focus of this thesis, because it predicts future risk of dementia (Amieva et al., 2005; M. F. Elias et al., 2000), represents the point at which interventions may be possible, affects a larger proportion of the population, and is independent of formal diagnosis, which is never entirely objective despite guidelines (e.g., Diagnostic and Statistical Manual of Mental Disorders, 4th edition (American Psychological Association, 1994)). Reflecting the notion that the entire cognitive continuum rather than thresholds for impairment should be investigated, one commentary recently argued that "the concept of dementia is obsolete" (Hachinski, 2008, p. 2172).

1.3.3 Theories of cognitive ageing

This section offers a brief summary of different accounts of age-related cognitive declines. Some centre on parallel declines or correlations between cognitive ability and physiological measures such as grip strength or sensory function (Anstey, Dear, Christensen, & Jorm, 2005; Clay et al., 2009; Grady & Craik, 2000; Li & Lindenberger, 2002) (although longitudinal studies have produced mixed evidence on this topic (Anstey, et al., 2005; Henderson et al., 2011)). The 'sensory deprivation hypothesis' suggests that such findings reflect effects of age-related 'sensory underload' restricting processing abilities and engagement in cognitively stimulating activities (Lindenberger & Baltes, 1994). Alternatively, a cognitive component of sensory functioning may increase in load with age (Baltes & Lindenberger, 1997). The 'common-cause hypothesis' assumes that age-related deterioration in the 'mechanics' of the physiological system causes both physiological and cognitive declines (Lindenberger & Baltes, 1994). These 'mechanics' may include factors uniquely related to the central nervous system (e.g., dopaminergic functioning) or the entire organism (Li & Lindenberger, 2002). Shared resources between cognitive and physiological measures (Li & Lindenberger, 2002) and similarities between cognitive and physical tasks such as dependence on the ability to follow task instructions (Salthouse, Hambrick, & McGuthry, 1998) are both also possible 'common causes'. Finally, correlations between cognitive and physiological measures may be artefacts of higher-ability individuals engaging in behaviour which ensures high sensory performance, such as bringing their spectacles to testing sessions (Henderson, et al., 2011).

Other accounts of cognitive ageing focus on age-related declines in attentional resources (Craik & Rose, 2012) or speed of processing (Salthouse, 1996). According to the latter, reduced speed of processing puts constraints on a ‘limited time mechanism’ which allows the use of information for only a limited time during complex tasks, as well as a ‘simultaneity mechanism’ (Clay, et al., 2009). Age-related increases in reaction time (Fozard, Vercruyssen, Reynolds, Hancock, & Quilter, 1994; Le Carret, Lafont, Mayo, & Fabrigoule, 2003), age-related delays in event-related potentials (ERP) during task performance (Dustman et al., 1990) and evidence for processing speed as a predictor or mediator for declines in other cognitive domains (Elgamal, Roy, & Sharratt, 2011; Finkel, Reynolds, McArdle, & Pedersen, 2007) may be seen to support this account of cognitive ageing.

In contrast, the ‘executive function’ or ‘frontal lobe hypothesis’ assumes age-related declines initially in frontal and prefrontal abilities as the bases for later disruption of other cognitive domains (Dempster, 1992). The specific abilities prone to decline may include the ability to strategically encode and retrieve stimuli, or to resist interference from distractors (Buckner, 2004). This account of cognitive ageing finds support in evidence of loss of inhibitor function, and reduced task switching and planning abilities with advanced age (Span, Ridderinkhof, & van der Molen, 2004).

Finally, the ‘depth of processing’ hypothesis suggests that processing becomes increasingly shallow due to under-recruitment of memory systems (Craik & Lockhart, 1972) and is, for instance, supported by reports of differences in hippocampal activation between younger and older individuals during deep versus shallow encoding tasks (Daselaar, Veltman, Rombouts, Raaijmakers, & Jonker, 2003). The ‘effortfulness hypothesis’ very similarly posits that age affects task demands, and is consistent with age-related declines in dual task performance (Tun, McCoy, & Wingfield, 2009), as well as links between age-related declines in cognitive performance and task complexity (Salthouse, 1992).

Because each account of cognitive ageing is supported by evidence, no one ‘correct’ version has been agreed on to date. Many of the theorists themselves acknowledge large overlaps between the accounts, and it is likely that cognitive ageing is a complex process with a number of different underlying mechanisms.

1.4 Pathological cognitive ageing

1.4.1 Impairment short of dementia

As aforementioned and illustrated in Figure 1.1, the onset of dementia is not often sudden - brain pathology and symptoms appear to develop over the course of decades. In the 1980s, the need to identify patients in the ‘grey areas’ or ‘transitional phases’ between ‘normative’ cognitive decline and dementia became apparent, because clinicians were often faced with patients who exhibited clear cognitive deficits but did not (yet) meet the diagnostic criteria for dementia (Geda, 2012). A pre-clinical phase of dementia was identified during which individuals either experience very subtle cognitive symptoms or are symptom-free but have biomarkers consistent with dementia (e.g., beta amyloid in cerebrospinal fluid) (Sperling et al., 2011). During another pre-dementia stage recently described as ‘subjective cognitive impairment’ symptoms begin to become apparent (Geda, 2012). This is then followed by a phase involving mild degrees of impairment. There is some disagreement in terms of terminology and diagnostic criteria for this phase. Widely used terms include cognitive impairment/ no dementia (CIND), age-associated memory impairment (AAMI) and mild cognitive impairment (MCI). CIND is commonly defined as impairment which does not meet criteria for dementia (irrespective of whether this impairment is in a memory or another cognitive domain) and often includes individuals with cognitive impairment due to other medical conditions, such as Parkinson’s disease. In contrast, AAMI typically rules out presence of other medical conditions and restricts impairment to a memory domain (Ward, Arrighi, Michels, & Cedarbaum, 2012).

MCI may be amnestic (involving memory impairment) or non-amnestic (without memory involvement). For amnestic MCI, a large proportion of the literature uses diagnostic criteria suggested by Petersen (1999). In absence of dementia, patients exhibit intact activities of daily living and normal general cognitive function, but have abnormal memory considering their age. For single-domain amnestic MCI, memory is the only domain affected; for multi-domain MCI several domains including memory are compromised (Geda, 2012). Additionally, patients must have a subjective memory complaint (R. C. Petersen, et al., 1999), although in practice, this criterion is often neglected (Ward, et al., 2012). Diagnosis made on the basis of a single assessment may also be complicated by the fact that some individuals may have long-standing memory problems which have not actually progressed up to the point of assessment (R. C. Petersen, et al., 1999). Prior to meeting the diagnostic criteria, MCI is usually preceded by detectable acceleration of cognitive decline (Sperling, et al., 2011).

Once diagnosis is made, between 1% and 26% of patients progress to dementia annually (R. C. Petersen, 2004). Increased brain pathology that is typical of Alzheimer's disease (Guillozet, Weintraub, Mash, & Mesulam, 2003) suggests that MCI may constitute a prodromal stage of Alzheimer's disease (Hedden & Gabrieli, 2004). Crucially, reversion to near-normal cognitive function is also possible in patients with MCI, particularly when patients are younger at the time of diagnosis (Koepsell & Monsell, 2012; Marioni et al., 2012).

1.4.2 Dementia

In contrast to MCI, recovery to near-normal functioning is impossible in dementia. The condition is associated not only with severe cognitive deficits, but also with behavioural and motor problems, overall contributing to a high risk of dependency and mortality. Dementia-typical symptoms were described as early as 2000 BC in Egyptian writings and were referred to as "being out of one's mind" in 50 BC. Yet, dementia was not recognised as a disease until the 18th century (Bourgeois & Hickey, 2009). Today, pharmaceutical treatments may offer symptomatic relief and decelerate disease progression, but a cure is yet to be found (Farlow, Miller, & Pejovic, 2008). It is also unclear whether or not dementia shares a common cause with normative age-related decline, or whether it has a distinct pathology (Buckner, 2004). Thirty-three % of women and 16% of men over the age of 65 are currently expected to develop dementia (Gregg et al., 2000). Due to an ageing society, global incidence may double between 2010 and 2050 (Gustafson, 2006). This development exerts severe pressures and "if left unchecked (…) will destroy the health care system" (The Alzheimer's Association in the USA, 2003; cited in (B. Wood, 2005, p. 258)). Dementia is an extremely heterogeneous condition (Etgen, Sander, Bickel, Sander, & Forstl, 2010). Each patient with dementia is unique depending on their level of dependence, support from carers, and demands faced in everyday life. On the basis of patterns of symptoms and brain pathology, different types of dementia have been identified, including Alzheimer's disease (AD), vascular dementia (VaD), fronto-temporal dementia (FTD), and dementia with Lewy bodies (DLB). Diagnosis is only certain following post-mortem examination, although even then precise guidelines on the extent of pathology required for diagnosis are lacked (J. A. Schneider, Arvanitakis, Bang, & Bennett, 2007). AD and VaD together account for around 80% of cases (J. A. Schneider, et al., 2007), although it is estimated that as many as 50% may be affected by mixed pathology (Woodward, Mackenzie, Hsiung, Jacova, & Feldman, 2010). Particularly the boundaries between VaD and AD pathology do not appear to be distinct. It has been suggested that a majority of AD in fact represents misdiagnosed VaD with a minimal component of AD (Román, 2005), or that any distinction between AD and VaD may be futile due to an atherosclerotic component of AD (Craft, 2009; Scheid & Voigt, 2005).

Alzheimer's disease (AD)

Initial symptoms of AD include naming difficulty and executive dysfunction (R. G. Morris, 2004), as well as deficits in memory and reasoning (M. F. Elias, et al., 2000; B. J. Small, Fratiglioni, Viitanen, Winblad, & Backman, 2000). A family history is associated with early disease onset in middle-age whereas risk factors for the more common form of late-onset AD are less clear. AD is characterised by synaptic loss, neuronal death, as well as high densities of extracellular neuritic senile plaques (SP) and intracellular neurofibrillary tangles (NFT) (Arendash & Cao, 2010; Woodward, et al., 2010), which are further described below. Disease progression has been described in six stages, with initial development of beta amyloid and subsequent development of NFT (H. Braak & Braak, 1991). This pathology at first contributes to a reduction in hippocampal volume and atrophy in medial temporal structures, and later to atrophy in the temporal neocortex, parietal and frontal lobes (T. C. Alves & Busatto, 2006). The specific causes of AD are debated. According to the amyloid cascade hypothesis, beta amyloid deposition in the brain initiates the development of NFTs and neuronal death (Hardy & Higgins, 1992). Symptoms may first arise when accumulated NFT disturb frontal circuits responsible for executive function (Román, 2005). Another view assumes that a decline in metabolic activity in the brain leads to both cognitive dysfunction and beta amyloid deposition without a causal relationship between the two (Struble, Ala, Patrylo, Brewer, & Yan, 2010). Environmental insults resulting in the degeneration of neurons and accumulation of cellular breakdown products, as well as increased production of inflammatory cytokines have also been implicated as possible causal factors (Armstrong, 2009; Clark, Atwood, Bowen, Paz-Filho, & Vissel, 2012). Finally, insulin levels appear to play a role in the deposition of beta amyloid, because both insulin and beta-amyloid compete for degradation by the insulin-degrading enzyme (IDE) (Bourdel-Marchasson, Lapre, Laksir, & Puget, 2010; Marlowe et al., 2006). This may contribute to the observation of an increased prevalence of (pre-)diabetes in AD (one US study found abnormal glucose levels in as many as 81% of AD patients (Janson et al., 2004)), and has led some to suggest the terms ‘type III diabetes’ (Lester-Coll et al., 2006), ‘neural diabetes’ (McNay & Recknagel, 2011) or ‘brain diabetes’ (Tomycz & Friedlander, 2011) to describe AD brain pathology.

Vascular dementia (VaD)

VaD is thought to be caused by cerebral micro- and macrovascular disease evident for example in ischemic or hemorrhagic strokes, micro-strokes and hyperperfusive ischemic injury (Román, 2005). Diagnostic criteria for VaD include memory loss and progressive impairment with detrimental effects on activities of daily living. Lesions in VaD are usually less localised compared with those seen in AD patients, and disease onset is relatively fast, with fluctuation in severity of symptoms (Erkinjuntti, 2007). Several subtypes of VaD have been identified. Large-vessel VaD with highly variable symptoms arises following multiple infarcts or strategic infarcts in cortical or subcortical area. Symptoms depend on the location of the infarct (Kalaria &

Erkinjuntti, 2006). VaD may also occur as subcortical ischaemic VaD, cortical-subcortical VaD, ischaemic-hypoperfusive VaD and haemorrhagic VaD (Erkinjuntti, 2007; Strachan, Reynolds, Frier, Mitchell, & Price, 2008). Subcortical VaD, for instance, is characterised by early deficits in attention, executive function, processing speed and motor slowing, with initially only mild memory impairments (Kalaria & Erkinjuntti, 2006).

Other types of dementia

Other types of dementia include dementia with Lewy bodies (DLB), characterised by presence of α-synuclein proteins, which may develop into neurotoxic fibrils, as well as AD-typical SP in the limbic and/or neocortex. Patients experience a fluctuation in cognitive ability, hallucinations, as well as visuospatial and motor dysfunction commonly associated with Parkinson’s disease (PD) (Cairns, 2004; Kahle, 2008; Woodward, et al., 2010; C.-K. Wu, 2011). In fronto-temporal dementia (FTD), which has been linked to genetic factors (Van Deerlin, Gill, Farmer, Trojanowski, & Lee, 2003), frontal and anterior temporal areas show symmetric bilateral atrophy (Neary & Snowden, 1996). Patients commonly exhibit changes in social conduct and personality, stereotypical behaviour, personal neglect, disinhibition, impulsivity, hyperactivity and PD-typical motor symptoms. Perceptual, language and spatial skills are relatively preserved (Neary & Snowden, 1996; Van Deerlin, et al., 2003). FTD is thought to be present in between 5% and 15% of dementia cases (Van Deerlin, et al., 2003).

1.5 Age-related changes in the brain

The cognitively unimpaired brain undergoes certain morphological and neurochemical changes during ageing, some of which are also typical of dementia. This section describes the typical hallmarks of brain ageing and their links to age-related cognitive declines.

1.5.1 Cerebral atrophy

Cerebral atrophy involves a reduction in neocortical thickness and neuron number, and is the most visible sign of brain ageing. The brain appears shrunk and loses weight and volume (Double et al., 1996; Enzinger et al., 2005). Brain atrophy has been linked to low level of cognitive function or cognitive decline in healthy older adults (Riley, Snowdon, & Markesbery, 2002), in older diabetes patients likely to suffer from type 2 diabetes (Akisaki et al., 2006; K. Hayashi et al., 2011; van Elderen et al., 2010) and in other patient populations (Egger et al., 2008; Lanz, Hahn, & Hildebrandt, 2007).

1.5.2 Cerebral vascular changes

Neuronal function in the brain is supported by a sophisticated vascular system which ensures the delivery of oxygen and nutrients. This system is apparent particularly in the white matter of the brain, which constitutes 40-50% of total brain volume. In unimpaired older adults, markers of integrity of the white matter have been found to correlate with cognitive ability (Kerchner et al., 2012), and some evidence suggests that processing speed may mediate this relationship (Deary, 2012).

Disease of the white matter is termed leukoaraiosis and includes white matter hyperintensities (WMH) caused by ischaemic lesions, white matter lesions (WMLs) and white matter atrophy (Madden, Bennett, & Song, 2009). Prevalence of leukoaraiosis increases with age (Longstreth et al., 1996; Salat et al., 2009). It is virtually always present in VaD but also in 9% to 19% of unimpaired older adults (Moretti et al., 2008). Presence of leukoaraiosis predicts the risk of future cerebral infarction and dementia, and has been linked to poor cognitive function and cognitive decline short of impairment in general older populations (de Groot et al., 2000; Debette & Markus, 2010; Verdelho et al., 2007) and in patients with type 2 diabetes (Reijmer et al., 2011). Cognitively impaired individuals may also experience faster advancement of leukoaraiosis compared with unimpaired individuals (Meyer, Rauch, Rauch, & Haque, 2000). The underlying mechanisms may involve cerebral infarctions or intracerebral haemorrhages (ICH) causing microvascular damage (for instance through interactions of intravascular fibrin with platelets and astrocyte swelling around capillaries and neurons), tissue damage, disruption to the integrity of the basal lamina and the blood-brain-barrier (BBB) (which is further described below), or alterations in cerebral blood flow (Petty & Wettstein, 2001). The importance of cerebral blood flow, for instance, which is high relative to other organs of the body (Moretti, et al., 2008), is evidenced in associations with cognitive decline and impairment (Kitagawa, 2010; Rombouts, Goekoop, Stam, Barkhof, & Scheltens, 2005; Ruitenberg et al., 2005).

1.5.3 Hippocampal changes

The hippocampus is an evolutionarily old structure in the medial temporal lobe and in humans is primarily responsible for declarative memory (J. J. Kim & Diamond, 2002). It is a site of neuronal plasticity and structural reorganisation, but is also vulnerable to ageing and stress (Artola, 2008). Small volume or atrophy of the hippocampus has been associated with poor cognitive function and cognitive decline including the presence of MCI and dementia (den Heijer et al., 2010; Golomb et al., 1994; Shi, Liu, Zhou, Yu, & Jiang, 2009). The different hippocampal regions vary in their degrees of vulnerability to age-related pathology. The CA1 subfield appears susceptible to vascular disease, the entorhinal cortex is affected in AD, and normative age effects are typically evident in the dentate gyrus (S. A. Small, Schobel, Buxton, Witter, & Barnes, 2011).

1.5.5 Neurofibrillary changes and senile plaques

Neurofibrillary tangles (NFT) are irregularly-shaped intraneuronal inclusions made up of paired helical filaments (PHF) and straight filaments (Armstrong, 2009). NFT occur both in the nerve cell soma and in the extracellular space as ‘ghost tangles’ which may result from the death and dissolution of an affected neuron and are eventually degraded by astrocytes (H. Braak & Braak, 1991; Gasparini, Terni, & Spillantini, 2007). Senile plaques (SP) develop from accumulated beta amyloids within a cell, and occur in pre-amyloid, neuritic and dense-cored forms (Armstrong, 2009). NFT and SP accumulate particularly in the hippocampus (Ryan & Geckle, 2000), and from a relatively young age. In one study, around 20% of individuals aged <30 years had some neurofibrillary changes in the brain. For over 90 year olds, this was the case for close to 100% (E. Braak et al., 1999). Presence and density of NFT and SP have been linked to cognitive impairment as well as low level of cognitive function in both cognitively impaired and unimpaired older adults (Arvanitakis et al., 2006; Guillozet, et al., 2003; Savva et al., 2009). In one relatively small study of cognitively healthy older adults and MCI patients, patterns consistent with NFT and SP on positron emission topography (PET) imaging also predicted rates of cognitive decline over the subsequent two years (G. W. Small et al., 2012).

Beta amyloid

Beta amyloid is a sticky, starchy plaque-like protein which develops from amyloid precursor protein (APP) (Rodrigue, Kennedy, & Park, 2009). Increased levels are the result either of an over-production or a failure of break-down processes due to a lack of beta-amyloid-degrading enzymes such as nephrilysin (NEP) and insulin-degrading enzyme (IDE) (Rodrigue, et al., 2009). Beta amyloid has synaptotoxic effects (Bell & Zlokovic, 2009) and in animal models leads to vascular oxidative stress and loss of vasodilatory function (Hamel, Nicolakakis, Aboulkassim, Ongali, & Tong, 2008). Beta amyloid imaged in PET has been negatively associated with cerebral blood flow (Buckner et al., 2005) and plasma beta amyloid may predict the risk of any dementia and AD in particular (Koyama et al., 2012). Amyloid is also accumulated during normal ageing. In one study, as many as half of 80 to 89 year olds with intact cognitive function had evidence of the protein in the brain (J. C. Morris et al., 2010). Overall, between 20% and 40% of older adults appear to have significant beta amyloid deposition (Jaworski et al., 2010), and particularly in medial prefrontal areas, the precuneus and posterior cingulate cortex (Rodrigue, et al., 2009). Evidence suggests associations of beta amyloid burden (determined in PET, cerebrospinal fluid or autopsy) with presence of MCI and AD, as well as with lower level of cognitive function in cognitively healthy older adults (DeCarli et al., 2012), although opposing results have also been reported. In two relatively small studies of healthy older adults and MCI patients, post-mortem beta amyloid burden was unrelated to cognitive test performance prior to death (Guillozet, et al., 2003; Mufson et al., 1999).

Tau

Tau is a phosphorylated protein with neurodegenerative effects. Accumulation occurs in neurons and neuroglial cells, such as oligodendrocytes and astrocytes. Tau is further present in PHF which contribute to NFT, as well as in isolated dendrites (neuropil threads) and in SP (Gasparini, et al., 2007). In contrast to beta amyloid, tau is present in all cases of AD (Jaworski, et al., 2010). Evidence of tau in cerebrospinal fluid or PET imaging has been linked to rate of cognitive decline and conversion to

AD in patients with MCI, and to the rate of cognitive decline in unimpaired older adults (Michell, 2009; Rolstad et al., 2013; G. W. Small, et al., 2012).

1.5.6 Changes to the blood-brain barrier

Brain and cerebrospinal fluid are separated from the circulating blood by the physical and chemical boundaries of the blood-brain barrier (BBB). The BBB allows passage of gaseous and small lipophilic molecules but prevents perfusion of components from the peripheral plasma, including red and white blood cells (Kojima et al., 2003; Zlokovic, 2008). Harmful molecules which breach the barrier may be actively exported (Shalev, Serlin, & Friedman, 2009).

The BBB was first demonstrated in 1909 (Goldmann, 1909; cited in (Zlokovic, 2008), but is still relatively poorly understood. Three layers have been identified: the cerebrovascular endothelium between blood and brain interstitial fluid, the choroid plexus epithelium and the arachnoid epithelium (Shalev, et al., 2009). Three types of 'junctions' are present in the endothelia. Gap junctions allow intercellular communication and the movement of solutes, ions and water between cells (Kojima, et al., 2003). Tight junctions (TJ) are formed by various proteins including occludin, claudins (the primary 'seal' of TJ), the junctional adhesion molecule (JAM-1) and accessory proteins such as membrane-associated guanylate kinae-like proteins or cingulin. Finally, adherens junctions (AJ) are formed by adhesion proteins of the cadherin family (Dejana, Orsenigo, & Lampugnanl, 2008; Hawkins & Davis, 2005). TJ and AJ have been likened to 'zippers' and 'fences', and take on gating functions at the BBB (Hawkins & Davis, 2005). Transport proteins (such as GLUT1 for the transport of glucose), receptor-mediated transcytosis or adsorptive transcytosis enable the passage of hydrophilic molecules (Abbott, Rönnbäck, & Hansson, 2006).

A breakdown by BBB function is characterised by the disruption of junctional components, transbarrier leakage, the secretion of immune factors, changes of astrocytes from a 'resting' to an 'active' status, necrotic changes in the endothelium and changes in the vascular basement membrane (Serlin, Levy, & Shalev, 2011; Shalev, et al., 2009). BBB functioning is altered in inflammatory states, hypoxia, cerebral ischemia (Hawkins & Davis, 2005; Wolburg et al., 2003), as well as in dementia and normative ageing (Farrall & Wardlaw, 2009). Links of BBB function with cognition are also evidenced in associations with cerebral blood flow (Hawkins & Davis, 2005). One way in which BBB function may affect cognitive function is through an influx of beta amyloid into the brain caused by interactions of the protein with RAGE on the BBB endothelium. The active transport of beta amyloid across the BBB into the peripheral blood may also be reduced when BBB function is compromised (Zlokovic, 2008).

1.6 Relative resistance to age-related pathology: cognitive and brain reserve

When investigating patterns of cognitive ageing, it is important to consider that an individual's late-life cognitive ability closely mirrors their peak pre-morbid ability achieved in young adulthood. In fact, between one quarter and one half of variance in ability remains stable over the course of the lifetime (Deary, 2012). Individual differences in pre-morbid ability also predict risk of late-life disease and mortality. Consistent with the findings of this relatively new field of 'cognitive epidemiology' (Deary & Der, 2005; cited in (Deary, 2012)), higher pre-morbid ability (McGurn, Deary, & Starr, 2008) and similarly high levels of education (Qiu, Bäckman, Winblad, Aguero-Torres, & Fratiglioni, 2001), high occupational complexity (Valenzuela & Sachdev, 2006), high socioeconomic status (SES) (Zeki Al Hazzouri et al., 2011) and high linguistic ability (Snowdon et al., 1996) are further related to reduced risk of cognitive impairment in later life, and particularly impairment due to vascular pathology (Deary, 2012). Although it is important to note that such findings are based on correlations and do not necessarily imply causation (Staff, 2012), these markers may be seen are proxies for a 'reserve capacity' protective of cognitive impairment. The relationship of a 'reserve capacity' with cognitive decline which is short of impairment is less clear. Whereas pre-morbid ability at age 11 did not predict the rate of late-life decline in the Lothian Birth Cohort of 1921 (Gow et al., 2008), it did in the Aberdeen Birth Cohort of 1921 and 1936 (Bourne, Fox, Deary, & Whalley, 2007), as well as in the National Survey of Health and Development (NSHD) (Gow et al., 2012). Late-life level of cognitive ability also predicted the rate of decline in the respective cognitive domain over annual examinations during six years of follow-up in the Religious Orders Study of non-demented Catholic clergy (Wilson, et al., 2002). However, due to occasional reports of even steeper cognitive declines in high- than in low-reserve individuals (Karlamangla et al., 2009; Singh-Manoux et al., 2011b), there is overall some controversy on the relationship between markers of reserve and the rate of cognitive decline short of impairment (Deary, 2012).

Individual differences in an ability to buffer a certain neuropathological burden may form the basis for the apparent individual differences in the reserve capacity which may affect vulnerability to cognitive ageing (Stern, 2006). For instance, it has been shown that up to 25% of older adults who are free of dementia prior to death in fact have neuropathological burden sufficient for diagnosis with AD (Neuropathology Group. Medical Research Council Cognitive Function and Aging Study, 2001). The individual differences in the capacity to preserve cognitive function may involve active or passive processes which have been conceptualised in different non-mutually exclusive accounts (Staff, 2012). The model of cognitive reserve (CR) emphasises active compensation for brain pathology (Stern, 2009). The brain counteracts age-related damage on the basis of its ‘neural reserve’, by engaging ‘software’ processes which upregulate pre-existing brain networks or cognitive paradigms (‘working harder’) (Staff, 2012). This may involve an increase in synaptic efficiency (Whalley, Deary, Appleton, & Starr, 2004), a more elaborate encoding of information, improved representation of the problem, application of knowledge of past consequences or previous solutions, and the application of algorithms which reduce processing requirements (Salthouse, 2003 cited in (Tucker-Drob, Johnson, & Jones, 2009)). Alternatively, the brain may switch to engaging novel brain networks or areas usually involved in higher-demand tasks during low-demand tasks, and thereby allow ‘neural compensation’ (‘doing different’) (Staff, 2012; Stern, 2002). Despite potentially equivalent anatomical bases, these kinds of compensatory processes would be more successful in a high-CR than in a low-CR individual. High-CR individuals would tolerate and compensate for a higher degree of damage and therefore reach the ‘point of inflection’, at which symptoms arise, only when a relatively higher neuropathological burden is reached (Stern, 2002, 2006, 2009).

In support of the CR account, high-reserve individuals are at higher risk of mortality and experience steeper cognitive decline once the ‘point of inflection’ is reached (Marioni, et al., 2012; Stern, 2006). Moreover, cerebral metabolism may be lower in high- than in low-reserve patients with dementia (Alexander et al., 1997). Networks recruited by high-reserve older adults may also differ from those recruited by younger adults with the same background (Stern et al., 2005), and animal studies have demonstrated beneficial effects of enriched environments (comparable to high SES) on neurogenesis, cell proliferation and cognitive performance (Veena et al., 2009). Overall, this evidence suggests a degree of active compensation and re-organisation of processing networks.

In contrast to CR, functional threshold models of brain reserve capacity (BRC) (Satz, 1993; cited in (Stern, 2009)) assume that brain damage caused by neuropathology affects all individuals in the same way, with no individual differences in functional compensation. Susceptibility to clinical expression is instead caused by individual differences in passive processes and the anatomical ‘hardware’ of the brain, which may include synapse counts and connectivity, brain size and neural density(Richards & Deary, 2005; Stern, 2006). Progressive neuropathology gradually decreases BRC, but clinical expression only occurs once BRC is depleted, at a fixed threshold or cut-off point that is identical for all individuals (Stern, 2006, 2009).

For instance, symptoms may occur when synapse counts are reduced to a specific number insufficient to sustain normal functioning (Stern, 2009). It follows that, rates of cognitive decline would be equivalent in individuals with higher and those with lower reserve capacity. Individuals with higher initial ability simply reach the threshold for cognitive deficits at later time points compared with low-ability individuals due to higher ‘starting points’(Tucker-Drob, et al., 2009). This type of model is unable to account for the aforementioned individual differences in recruitment of brain networks (Stern, 2009), but finds support in evidence of higher

BRC-related markers such as brain size or neuronal density in individuals with high reserve (e.g., high pre-morbid ability; high SES) as well as in the aforementioned individuals with dementia-typical pathology who remain symptom-free (Staff, 2012). Given that both CR and BRC models are supported by evidence and that their distinctions are hardly clear-cut (Stern, 2009), it may be likely that both play a role in determining individuals' susceptibility to cognitive ageing. In line with this, Richards and Deary (2005) recently proposed a dynamic lifecourse model of cognitive reserve, which incorporates the 'software' and the 'hardware' components of CR and BRC and further takes into account environmental, behavioural and genetic influences.

1.7 Cognition in diabetes

1.7.1 Brain pathology in diabetes

The brains of patients who suffer from diabetes tend to have increased vulnerability to age-related pathology compared with those of healthy older adults. For instance, relative to non-diabetic controls, cerebral atrophy has been found to be more severe in older adults with type 2 or any type of diabetes (Kumar, Anstey, Cherbuin, Wen, & Sachdev, 2008; van Elderen, et al., 2010). A meta-analysis of brain imaging studies supported these observations and additionally established links of any type of diabetes with cerebral macrovascular disease evident in lacunar infarcts, as well as with regional alterations in cerebral blood flow (van Harten, De Leeuw, Weinstein, Scheltens, & Biessels, 2006). Diabetes patients are further prone to cerebral microvascular damage (Mankovsky, Metzger, Molitch, & Biller, 1996).

Despite the aforementioned link of insulin with beta amyloid deposition (Bourdel-Marchasson, et al., 2010), the evidence for AD-typical pathology in diabetes patients is somewhat less consistent. For instance, one small Spanish study reports more severe AD-typical pathology in AD patients with diabetes compared with non-diabetic AD patients (Valente, Gella, Fernàndez-Busquets, Unzeta, & Durany, 2010), whereas such results were not found in the relatively larger US-based Religious Orders Study or in another US study, which compared general older patients with type 2 diabetes and diabetes of any type respectively to non-diabetic controls (Arvanitakis, et al., 2006; Janson, et al., 2004) .

The hippocampus is the structure of the brain which appears to be most vulnerable to effects from disturbed glucose metabolism. Here, type 2 diabetes (Bruehl, Wolf, & Convit, 2009; K. Hayashi, et al., 2011) and even reduced glucose tolerance in non-diabetic older adults (Convit, Wolf, Tarshish, & de Leon, 2003) have been associated with increased atrophy and reduced volume compared with non-diabetic and normoglycaemic controls, respectively. Hippocampal function may also be altered through a shift in postsynaptic depolarisation inhibiting long-term potentiation and facilitating long-term depression (Artola, 2008).

Moreover, diabetes patients may suffer from disruption in blood-brain barrier (BBB) function. In rodents, the condition has also been associated with increased permeability, reduced occludin content and reduced occludin immunoreactivity in the BBB or the blood-retinal barrier which is thought to closely resemble the BBB (Antonetti et al., 1998; Barber, Antonetti, & Gardner, 2000; Hawkins, Lundeen, Norwood, Brooks, & Egleton, 2007).

Patients with diabetes are further exposed to increased levels of oxidative stress resulting from increased formation of reactive oxygen species (ROS) and/or reduced levels of antioxidant scavengers. Oxidative stress contributes to damage of lipids, membranes, proteins and DNA (Rains & Jain, 2011). These processes, which are likely to equally affect the body and the brain, then result in endothelial dysfunction, vascular disease and the formation of advanced glycation endproducts (Pitocco et al., 2010). Overall, the evidence suggests that many of the aforementioned normative age-related changes in the brain are more severely or more commonly expressed in patients affected by diabetes.

1.7.2 Associations of diabetes with reduced cognitive function short of dementia

Given the evidence for structural differences between the brains of individuals with and those without diabetes, associations of the disease with brain function are unsurprising. Such links were initially noted in the 17th century (Willis, 1684; cited in (Coker & Shumaker, 2003)), but were disregarded until the first half of the 20th century (Miles & Root, 1922). Since then, cross-sectional case-control studies of diabetic individuals (presumably mainly of type 2) and non-diabetic older individuals have reported reduced levels of global cognitive ability, as well as reduced memory and visuospatial ability, poorer executive function and slower processing speed, evident for instance in longer latencies on reaction time tasks in people with diabetes (Arvanitakis, Wilson, Bienias, Evans, & Bennett, 2004; Colberg, Somma, & Sechrist, 2008; Grodstein, Chen, Wilson, & Manson, 2001; Richerson, Robinson, & Shum, 2005; van den Berg, de Craen, Biessels, Gussekloo, & Westendorp, 2006), despite occasional null results (Arvanitakis, et al., 2004; Perlmutter et al., 1984; Rodrígyez-Saldana et al., 2002). In support of diabetes-associated cognitive deficits, diabetes has also been linked to longer latencies and lower amplitudes in the P300 component of event-related potentials (ERP) thought to reflect cognitive processing (Andreadou, Mitrakou, Constantinides, & Triantafyllou, 2012; de Freitas Alvarenga et al., 2005). Numerous cohort studies (and more rarely, randomised controlled trials), which prospectively observe trajectories of cognitive decline over at least two (but usually more) years of follow-up in community-dwelling subjects, have been performed to assess diabetes associations with cognitive decline short of dementia. The findings of the key studies are summarised in Table 1.1. Prevalent or incident diabetes (presumably mainly of type 2) has been associated with an acceleration of decline in global ability (Gregg, et al., 2000; Hassing et al., 2004; Tilvis et al., 2004; Yaffe et al., 2012), processing speed (Arvanitakis, et al., 2004; Fontbonne, Berr, Ducimetière, & Alpérovitch, 2001; Gregg, et al., 2000; Knopman et al., 2001; Spauwen, Köhler, Verhey, Stehouwer, & van boxtel, 2013; Yaffe, et al., 2012), verbal and non-verbal memory (Fontbonne, et al., 2001; Spauwen, et al., 2013) and executive function (Fontbonne, et al., 2001; Gregg, et al., 2000; Kanaya, Barrett-Connor, Gildengorin, & Yaffe, 2004; Knopman, et al., 2001; Spauwen, et al., 2013; van Elderen, et al., 2010). Occasionally, equivalent rates of cognitive declines have been reported for subjects with prevalent or incident diabetes and non-diabetics (Arvanitakis, et al., 2004; Fontbonne, et al., 2001; Kanaya, et al., 2004; van den Berg, et al., 2006; J. H. Wu et al., 2003; Yaffe, et al., 2012), or for individuals with IFG and normoglycaemic controls (Rouch et al., 2012).

Table 1.1: Prospective evidence linking diabetes with cognitive decline short of dementia

Study	Sample	Design	Total n	Age at baseline	Prevalence of diabetes	Measurement of cognitive function	Covariates in final models	Main finding in fully adjusted models
Gregg et al. (2000)	Cohort of females	3 to 6-year prospective	9679	65–99 years (mean 72)	682 (7.0%); any type of diabetes	MMSE, TMT-B, DSC	Age, education, depression, visual impairment, stroke, hypertension, estrogen use, smoking, self-rated health	Diabetes patients declined more steeply on DSC and MMSE compared with others (for TMT-B p=0.06)
Fontbonne et al. (2001)	Epidemiology of Vascular Aging Study (all MMSE>26 at baseline)	4-year prospective	961	59–74 years (mean 65)	55 (5.72%); any type of diabetes	MMSE, large test battery. 'Serious worsening' defined as change into bottom 15% of distribution	Age, sex, education, blood pressure, BMI	Diabetes patients at increased risk of 'serious worsening' in verbal learning/ DSC/ Faces/ finger tapping when adjusted for covariates except BMI. Only association with finger tapping survived adjustment for BMI
Knopman et al. (2001)	Atherosclerosis Risk in Communities (ARIC)	Mean 6-year prospective	10 963	47–70 years (mean 57)	1329 (12.12%); any type of diabetes	Large battery	Age, sex, assessment centre, education, medication relevant to central nervous system	Diabetes patients declined more steeply on DSC and verbal fluency

Wu et al. (2003)	SALSA project of US Latinos	2-year prospective	1789	>60 years (mean 71)	585(37%); any type of diabetes	MMSE, word recall. 'Major decline' defined as worst 10th percentile of change	Age, sex, education, depression, acculturation, hypertension	1. Diabetes patients declined more steeply on MMSE/ word recall 2. Diabetes patients at increased risk of 'major decline' on MMSE 3. No relationship with major decline on word recall.
Arvanitakis et al. (2004)	Religious Orders Study	Up to 9-year prospective	824	>55 years (mean 75)	15.4%; any type of diabetes	MMSE, large test battery. Calculated 'summary scores' for each cognitive domain from z-scores	Age, sex, education	1. Diabetes patients declined more steeply in perceptual speed (at additional 0.3SD/year) 2. No relationship between diabetes status and decline in global function (p=0.06) or other cognitive domains.
Hassing et al. (2004)	OCTO-Twin Study (all MMSE>23 at baseline)	6-year prospective	258	>80 years (mean 83)	38 (14.73%); any type of diabetes	MMSE	Age, sex, education, smoking, cardiovascular disease, cerebrovascular disease	Diabetes patients declined more steeply on MMSE
Kanaya et al. (2004)	Rancho Bernardo Study	4-year prospective	999	42–89 years (mean 69 years for non-diabetics; 74 years for diabetics)	118 (11.81%); any type of diabetes	MMSE, verbal fluency, TMT-B. 'Major decline' defined as worst 10th percentile of change	Age, sex, depression, APOE e4, oestrogen use	Diabetes patients (females only) at increased risk of 'major decline' in verbal fluency. No other significant findings.

Tilvis et al. (2004)	Helsinki Aging Study	Up to 10-year prospective	650	>75 years	101 (15.54%); any type of diabetes interpreted as type 2	'Decline' defined as 4-point decline on MMSE or progression to lower CDR class	Age, sex	1. Diabetes patients at increased risk of 'decline' over 5 years (RR 2.2) 2. Diabetes status unrelated to risk over 10 years
Van den Berg et al. (2006)	Leiden 85+ study	5-year prospective	596	85 year olds	96 (16.11%); any type of diabetes	MMSE, DSC, word learning, Stroop	Sex, education	Diabetes patients declined more steeply on MMSE/ DSC/ word learning, but not Stroop
Van Elderen et al. (2010)	PROSPER study (individuals with vascular disease or at risk of vascular disease)	3-year RCT	527	70–89 years (mean 75)	89 (16.89%); any type of diabetes	Stroop, DSC, picture learning	Age, sex, education, hypertension, treatment	Diabetes patients declined more steeply on Stroop and picture learning (immediate but not delayed)
Rouch et al. (2012)	PROOF study	2-year prospective	163	Mean 66 years	26 (15.95%) had IFG; 12 (7.34%) had any type of diabetes	TMT-A, TMT-B, Stroop, DSC, Benton Visual Retention Test, forward and backward digit span, category fluency, letter fluency, FCSR	Age, sex, education, anxiety symptoms, depressive symtpoms, smoking, cholesterol, triglycerides, BMI, blood pressure	Diabetes at baseline related to steeper decline on TMT-B and Stroop; IFG related to steeper decline on letter fluency
Yaffe et al. (2012)	Health, Aging and Body Composition Study (Health ABC)	9-year prospective	3069	70–79 years (mean 74)	717 (23.4%); any type of diabetes	MMSE, DSC	Age, sex, education, race	1. Diabetes at baseline related to steeper decline on DSC/ MMSE. 2. No relationship between incident diabetes and

								cognitive decline.
Spauwen et al. (2013)	Maastricht Aging Study	6-year and 12-year prospective	1290	40–83 years (means 59 years for non-diabetics; 69 years for diabetics)	63 (5.8%); type 2 diabetes	MMSE, verbal learning, concept shifting, DSC	Demographics, smoking, alcohol, hypertension, cardiovascular disease, BMI, depression	1. In crude analysis: baseline diabetes related to 3-fold steeper decline on DSC and 4-fold steeper decline in executive function over 6 and 12 years. 2. Findings attenuated in fully adjusted models. 3. No relationship between incident diabetes and cognitive decline.

DSC, Digit Symbol Coding; MMSE, Mini-Mental State Examination; TMT-B, Trail-Making Test-B; CDR, Clinical Dementia Rating Scale; BMI, body mass index; RR, relative risk; IFG, impaired fasting glucose; FCSR, Free and Cued Selective Reminding Test; APOE e4, apolipoprotein e4 allele.

A systematic review of prospective studies determined an overall 1.2 to 1.5-fold accelerated rate of cognitive decline in older adults with any type of diabetes which are presumably mainly affected by type 2 diabetes (Cukierman, Gerstein, & Williamson, 2005). Similarly, younger adults with type 1 diabetes typically exhibit cognitive deficits when compared with age-matched controls (Brands, Biessels, De Haan, Kappelle, & Kessels, 2005). Mainly processing speed appears to be compromised in diabetes (McCrimmon, Ryan, & Frier, 2012). Type 2 diabetes in particular is additionally associated with deficiencies of moderate effect size in verbal memory, attention and executive function (Reijmer, van den Berg, Ruis, Kappelle, & Biessels, 2010).

Patients with type 2 diabetes are also at increased risk of diagnosis with MCI (Bourdel-Marchasson, et al., 2010) and of cognitive impairment short of dementia which is defined using less robust measures. For instance, compared to non-diabetics, patients have been found to be more likely to score in the lowest tenth percentile of cognitive test distributions (Gregg, et al., 2000) or below the cut-point <24 on the Mini-Mental-State Examination (MMSE) commonly applied to identify cognitively impaired individuals (Cartwright et al., 2012). Conversely, prevalence of impairment measured in this way may be raised in diabetic populations. For instance, 20% of over 80 year olds participating in the Fremantle Cognition in Diabetes Study set in Australia obtained MMSE<24 (Bruce, et al., 2003) compared with prevalence of between 5% and 16% in the general older (80-84 years) Australian population (Anstey et al., 2010).

1.7.3 Associations of diabetes with dementia

In terms of clinically diagnosed dementia, baseline diabetes (presumably mainly of type 2) predicted 65% increased risk of Alzheimer's Disease (AD) over six-year follow-up in the Religious Orders Study (Arvanitakis, et al., 2004), in Taiwan predicted a 63% increased risk of any dementia over 7 years of follow-up (Cheng, Sy, Wu, Wang, & Chen, 2012), and in an early US study increased risk of any dementia by 66% over follow-ups of between 0 and 15 years (Leibson et al., 1997). Others report even larger effect sizes. In the Rotterdam Study, risk of any form of dementia was almost two-fold in type 2 diabetes patients compared with non-diabetic controls (Ott et al., 1999). Similarly, patients with type 2 diabetes had relative risks of 1.8 for AD and 2.3 for vascular dementia (VaD) in the Honolulu-Asia Aging Study (Peila, Rodriguez, & Launer, 2002). In a Canadian cohort, diabetes (presumably mainly of type 2) also predicted a two-fold five-year risk of VaD, although associations were not found for AD or any dementia (MacKnight, Rockwood, Awalt, & McDowell, 2002). In contrast, an almost five-fold increased risk of incident AD in 70-year old diabetes patients (presumably mainly of type 2) compared with non-diabetic controls over a mean of 13 years of follow-up were found in the Framingham Study (Akomolafe et al., 2006). Oddly, one study established a reduced rate of two-year global decline in older diabetics with AD when compared to non-diabetics with AD (Sanz, Andrieu, Sinclair, Hanaire, & Vellas, 2009).

A systematic review of cohort studies with prospective designs established a relative risk for any form of dementia of 1.5 and of 2.4 for VaD in older patients with diagnosed diabetes (which are presumably mainly of type 2) compared with non-diabetic controls (Lu, Lin, & Kuo, 2009). Another systematic review (which also grouped type 1 and type 2 diabetes together) reports similar findings, with a risk of 1.6 for any dementia (Cukierman, et al., 2005). The apparent larger effect sizes in associations for VaD than for any dementia could potentially reflect the aforementioned inconsistency in evidence for AD-like pathology contrasting the relatively well-established cerebral vascular damage in patients with diabetes. Overall, 6% to 8% of dementia risk in over 65 year olds has been attributed to diabetes (Kloppenborg, van den Berg, Kappelle, & Biessels, 2008), and this observation may not be restricted to diagnosed diabetes. In a study of non-diabetic older adults, early insulin response at baseline predicted risk of AD over twelve years of follow-up (Rönnemaa et al., 2009).

At this point is should also be noted that although a majority of epidemiological research has focused on the investigation of selected clinical risk factors potentially contributing to the increased risk of cognitive impairment in people with type 2 diabetes (which is reviewed in the next chapter), some evidence suggests that diabetes-associated cognitive impairment may be driven by reverse causation. In a recent analysis of the Lothian Birth Cohort 1936, participants with diabetes had lower late-life cognitive ability short of dementia compared with the remaining subjects. However, the same individuals had also been tested for cognitive ability at age 11, and associations of diabetes with late-life ability disappeared following adjustment for childhood ability. Individuals with relatively lower cognitive ability at age 11 were therefore at increased risk of diabetes in later life, and had also carried their level of cognitive ability forward into later life (Mõttus, Luciano, Starr, & Deary, 2013). Similar findings were previously made in a relatively larger Dutch study. Here, participants with and those without diabetes were found to differ on four of six cognitive domains, but only the group differences in memory survived the adjustment for a vocabulary-based estimate of peak pre-morbid ability (Ruis et al., 2009).

To summarise, the literature provides robust evidence for an increased risk of cognitive impairment and accelerated cognitive decline in older patients with type 2 diabetes. Cognitive function appears to have increased vulnerability to diabetes during the 'crucial periods' during which the brain undergoes change, i.e. in early adulthood and later life (Biessels, Deary, & Ryan, 2008). Cognitive deficits in early adulthood may also aid to refute a recent suggestion that associations between diabetes and cognitive dysfunction are plausible on statistical grounds alone, due to increasing prevalence of both conditions with age (Brito, 2009). Although, some recent evidence suggests that links of diabetes and cognitive impairment for patients with type 2 diabetes in particular may be confounded by reverse causation which predisposes low-ability individuals both to late-life diabetes and late-life cognitive impairment, a number of clinical risk factors which could potentially also contribute to the observation have been identified. These are presented in the next chapter.

Chapter 2: Review of risk factors for cognitive decline

This chapter presents evidence on risk factors for age-related cognitive decline. Although a few key risk factors relevant to type 2 diabetes are selected for analyses in the thesis, a general overview of potential risk factors is presented here. Most are inter-related and may be associated with cognitive decline through various dynamic pathophysiological pathways involving causation, mediation, interaction and cumulation of effects (previously referred to as a “chain of risks” (Anstey, 2008, p. 284), but for the purposes of this review, each is considered individually in terms of their association with cognition, with a focus on populations with type 2 diabetes. Where evidence from populations with type 2 diabetes is limited, the literature on patients with type 1 diabetes or healthy older adults is also considered. The literature overall is inconsistent with respect to terminology and the use of cognitive instruments, with many studies relying on brief screening instruments for dementia (e.g., Mini-Mental State Examination, MMSE) despite potential unsuitability to detect subtle cognitive deficits (Tombaugh & McIntyre, 1992). Yet, any cognitive test, including screening instruments such as the MMSE, are considered equivalent for the purpose of this review. Because the various stages of age-related cognitive decline described in Chapter 1 are all seen to be part of the same process of cognitive ageing, these are also all reviewed together. The evidence for risk factor associations with dementia are described separately, because in contrast to cognitive decline short of dementia it is a clearly defined disease.

2.1 Metabolic risk factors

2.1.1 Obesity

Obesity refers to ‘above normal’ accumulation of body fat, which may have adverse effects on human health. Obesity is variously measured using body weight, body mass index (ratio of body weight to height with a BMI>30 often used to categorise ‘obese’ individuals (U. S. Department of Agriculture and U. S. Department of Health and Human Services, 2010)) or, less frequently, proportion of body fat. The accurate assessment of body fat using BMI or body weight is problematic, especially in older people, due to a higher rate of weight loss in heavy than in lighter individuals (D. K. Johnson, Wilkins, & Morris, 2006), age-related increases in adipose tissue in the absence of weight gain (Han et al., 2009), and because these measures do not capture the distribution of adipose tissue despite the fact that abdominal (or central, visceral) obesity is particularly linked to poor health outcomes (Boyko, Fujimoto, Leonetti, & Newell-Morris, 2000; Huxley, Mendis, Zheleznyakov, Reddy, & Chan, 2010). Attempts have been made to quantify abdominal obesity using the ratio of waist circumference to hip circumference, or waist-to-hip ratio (WHR>0.90 in males and >0.85 in females), with some success (Sundell, 2005). The prevalence of obesity is increasing, especially in developed countries. In the US, 13% of adults were obese at the beginning of the 20th century, compared with 33% in the 1960s (Ogden, Yanovski, Carroll, & Flegal, 2007). In Europe, current prevalence is between 4 and 28% for women and between 6 and 37% for men (Berghöfer et al., 2008). Around 10% of obese individuals suffer from type 2 diabetes (Sundell, 2005), and 85% of diabetes patients living in Scotland are overweight (BMI>25) (Wild & Byrne, 2006).

Obesity and cognition in the general population

In older people selected from the general population, obesity has been associated with reduced levels of performance on detailed neuropsychological tests (Dore, Elias, Robbins, Elias, & Nagy, 2009; Gunstad, Lhotsky, Wendell, Ferrucci, & Zonderman, 2010; Kilander, Nyman, Boberg, & Lithell, 1997). An association particularly with abdominal obesity has been supported by one study of over 8000 older females in which links between BMI and cognitive test performance were limited to high-WHR individuals (Kerwin et al., 2010). Some evidence suggests a potential for confounding of cross-sectional links by initial pre-morbid ability. In a Scottish birth cohort followed between age 11 and later life, late-life BMI correlated inversely both with ability at age 11 and with two estimates of peak pre-morbid ability. Additional associations between BMI and level of cognitive test performance aged 70 became non-significant when ability at age 11 or social class were controlled for, potentially demonstrating causality in the direction of cognitive ability-body weight (Corley, Gow, Starr, & Deary, 2010).

Yet, prospective investigations support a possible effect of obesity on subsequent cognitive decline and even on the development of brain atrophy (M. F. Elias, Elias, Sullivan, Wolf, & D'Agostino, 2003, 2005; Enzinger, et al., 2005; Gunstad, et al., 2010; Han, et al., 2009). In one relatively large-scale analysis of hospital records, midlife obesity was associated with a 74% increased risk of later dementia (Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005), although a review points out that this may be due to obese individuals having more contact with medical services and therefore being more likely to receive dementia diagnosis (Lawlor, Lean, & Sattar, 2006). Systematic reviews on this topic suggest a small to moderately-sized effect of obesity on increased risk of dementia and cognitive decline that is short of dementia (Profenno, Porsteinsson, & Faraone, 2010; van den Berg, Kloppenborg, Kessels, Kappelle, & Biessels, 2009).

Such observations may be related to the release of free fatty acids (Qin, Knol, Corpeleijn, & Stolk, 2010), adipokines leptin, adiponectin and resistin, as well as cortisol, oestrogen, and inflammatory cytokines from adipose tissue (Abbatecola et al., 2010), some of which may pass the blood-brain barrier (Sundell, 2005). Obesity is further associated with poor nutritional habits and resulting hyperglycaemia and hyperinsulinemia (Sundell, 2005), and it functions as marker of systemic vascular disease (Lawlor, et al., 2006).

Obesity and cognition in people with diabetes

In people with type 2 diabetes, high abdominal obesity or body fat levels have also been linked to poorer late-life cognitive ability short of dementia (Abbatecola, et al., 2010; E. Kim et al., 2008). In one of the investigations, the highest (versus lowest) tertile of total body fat mass at baseline was linked to a 68% increased risk of reduced cognitive function (MMSE<24) after two years of follow-up (Abbatecola, et al., 2010).

Despite the overall body of evidence suggesting a detrimental effect of obesity on cognition, a few studies have demonstrated positive correlations between BMI and cognitive test performance (Gunstad, et al., 2010; Onem et al., 2009) or apparent protective effects of baseline overweight on cognitive ability and decline in the general population and in people with diabetes (Kuo et al., 2006; Sturman et al., 2008; Thilers, Macdonald, Nilsson, & Herlitz, 2010). For instance, in the Fremantle Diabetes Study of older adults with type 2 diabetes, 76% of individuals with normal cognitive function at eight-year follow-up had had central overweight or central obesity at baseline, whereas this applied to only 62% of participants with reduced cognitive function short of dementia and to 61% of participants with dementia diagnosis by the time of follow-up (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008). The apparent duality of beneficial and detrimental effects of body weight has been described for a number of other health outcomes in diabetic and the general population (Doehner et al., 2011; Fogelholm, 2010; Sohn et al., 2012) as an 'obesity paradox' (Florez & Castillo-Florez, 2012), although the positive correlations with of body weight and cognition in particular could potentially reflect weight loss as a consequence of early stages of dementia (Whitmer, 2007). Overall, the relationship between obesity and cognition appears to be complex.

2.1.2 Dyslipidemia

Dyslipidaemia is generally characterised by high levels of total cholesterol and triglycerides, low levels of circulating high-density lipoprotein (HDL) and raised levels of low-density lipoprotein (LDL) cholesterol and/or low HDL:LDL ratio. HDL cholesterol has protective effects on the body in terms of the development of vascular disease, whereas LDL and total cholesterol have detrimental effects, although specific associations with health outcomes may be complex (Burillo et al., 2010; L. K. Petersen, Christensen, & Kragstrup, 2010).

Dyslipidemia and cognition in the general population

High midlife cholesterol has been associated with lower cognitive ability short of dementia in later life as well as increased risk of dementia (Kivipelto et al., 2002; Polidori, Pientka, Nelles, & Griffiths, 2010), but the evidence is less clear for late-life cholesterol. Some studies have reported higher cholesterol levels or a decreased rate of cholesterol absorption in individuals with poorer late-life cognitive test performance, in those with steeper cognitive decline, in those at increased risk of dementia (Stampfer, 2006; van den Kommer et al., 2011), and in individuals with hippocampal atrophy (Wolf et al., 2004). Cholesterol may be transported across the blood-brain barrier (Polidori, et al., 2010), so that the basis for associations of cholesterol with poorer cognitive function may involve effects of lipids on increases in oxidative stress (Polidori, et al., 2010) and beta amyloid deposition in the brain (González-Gross, Ascensión, & Pietrzik, 2001). Raised cholesterol has further been associated with microvascular and macrovascular damage (González-Gross, et al., 2001; Libby, Ridker, & Maeri, 2002). However, numerous other studies have failed to establish links between dyslipidemia and cognitive function in people with dementia or in older cohorts of the general population (den Heijer, Hofman, Koudstaal, & Breteler, 2005; Gillum & Obisesan, 2011; Orengo et al., 1996).

Dyslipidemia and cognition in people with diabetes

Although diabetes is characterised by high triglycerides, high LDL and low HDL (Ness, Aronow, & Ahn, 2000), studies of cholesterol and cognition in exclusively diabetic populations are rare. One small study of middle-aged to older type 2 diabetes patients reported similar cholesterol levels in individuals with and those without reduced cognitive ability short of dementia (R. H. Chen et al., 2012), although an earlier investigation of a similar population had established cross-sectionally significantly lower processing speed, reaction time and working memory in individuals with higher compared with those with lower triglyceride levels with medium to large effect sizes of associations (Cohen's d=0.33 to 0.65) (J. Cohen, 1992; Perlmutter et al., 1988).

Because cholesterol levels are modifiable, a number of randomised controlled trials (RCTs) have been carried out on the topic in the general population, but a recent review of the evidence concludes that a reduction in cholesterol does not appear to positively impact late-life cognitive function (van Vliet, 2012), overall leading to lack of consensus on the role of cholesterol in human cognition (Stampfer, 2006; van Vliet, 2012).

2.1.3 Hyperinsulinaemia

Insulin is a growth promoting hormone secreted from the pancreas in response to food intake and regulates glucose metabolism (Lamport, Lawton, Mansfield, & Dye, 2009). Through binding to receptors on the cell surface, particularly in skeletal muscle, insulin enables the transport of glucose through translocation of the glucose transporter 4 (GLUT4) to the plasma membrane and transverse tubules (Choi & Kim, 2010). Insulin resistance occurs when the normal response to insulin is reduced and higher levels of insulin are then required for normal glucose uptake (Clark, et al., 2012). Hyperinsulinaemia and insulin resistance have been linked to poor lifestyle, hyperglycaemia and hypercholesterolemia (Mangrum & Bakris, 1997; Tomycz & Friedlander, 2011), and consequently have been associated with poor health outcomes even in non-diabetic populations (Ausk, Boyko, & Ionnou, 2010).

Insulin and cognition in the general population and in people with diabetes

Exogenous insulin has been shown to improve cognitive function at least in the short term and with sufficient glucose availability (Bourdel-Marchasson, et al., 2010). Yet, insulin resistance or chronically elevated endogenous levels (which are common in type 2 diabetes due to initial enhanced production in response to insulin resistance) have been linked to poorer late-life cognitive function short of dementia and steeper cognitive decline in observational studies of the general population and of people with diabetes (Bruehl, Sweat, Hassenstab, Polyakov, & Convit, 2010; Geroldi et al., 2005; Heyer, Mergeche, Bruce, & Connolly, 2013; Kalmijn, Janssen, Pols, Lamberts, & Breteler, 2000; Kuusisto et al., 1993; Umegaki et al., 2008; Young, Mainous, & Carnemolla, 2006). In the Honolulu-Asia Aging Study, individuals in the highest 15th percentile of the baseline distribution of insulin had a 54% increased risk of dementia five years later (Peila, Rodriguez, White, & Launer, 2004). Null results have also been reported, however (Euser et al., 2010; Isik et al., 2007), and a systematic review of studies including both diabetic and non-diabetic individuals concluded that the observational evidence for an association of hyperinsulinaemia and/or insulin resistance with poor cognitive outcome was inconclusive (Lamport, et al., 2009). The observation that the risk of dementia is greater for insulin-treated than for orally or dietary treated type 2 diabetes patients (Ott, et al., 1999) may well be confounded by factors typically related to insulin treatment, such as disease stage. Results from intervention studies suggest that a reduction in insulin levels may improve cognitive function in older patients with diabetes (Yanagawa et al., 2011) and decelerate cognitive decline in diabetic and non-diabetic patients with AD (Plastino et al., 2010;

Reger et al., 2008). This could be seen to suggest causal effects of hyperinsulinaemia on cognitive function. Insulin is actively transported crosses the blood-brain barrier into the brain, where it interacts with widely expressed receptors (Ryan & Geckle, 2000) and affects synaptic plasticity (McNay & Recknagel, 2011). Insulin further stands in relation to cortisol levels (McNay & Recknagel, 2011), and competes with beta amyloid for degradation by the insulin-degrading enzyme (IDE), so that higher insulin levels may indirectly lead to higher beta amyloid levels (Bourdel-Marchasson, et al., 2010; Marlowe, et al., 2006). However, the aforementioned beneficial effects of a reduction in insulin levels could potentially also arise due to beneficial effects of improved glycaemic control on cognition (described below).

2.1.4 Hyperglycaemia

The brain has high energy requirements, but is unable to synthesise glucose. Its glycogen supply also lasts only for a few minutes (Cryer, Davis, & Shamoon, 2003). Consequently, glucose consumption enhances cognitive performance in the short-term (Greenwood, Kaplan, Hebblethwaite, & Jenkins, 2003). To maximise performance, different areas of the brain are able to control their glucose levels locally (McNay, Fries, & Gold, 2000). Yet, in the short-term, abnormally elevated blood glucose levels, as present in diabetic ketoacidosis (DKA), can have severe detrimental effects on the brain. In one study of type 2 diabetes patients, experimentally induced hyperglycaemia (16.5mmol/l) reduced cognitive test performance compared to euglycaemic states in the same individuals. Effect sizes ranged between small and large for the individual cognitive tests, with hyperglycaemia accounting for between 2% and 56% of variance in performance, the largest of which was found for a reaction time task (Sommerfield, Deary, & Frier, 2004). Consequently, diurnal intra-individual fluctuations in glucose levels appear to be linked to fluctuations in cognitive function in this type of patient (McCall, 2005; Rizzo et al., 2010).

Chronic hyperglycaemia and cognition in the general population

Evidence suggests similarly detrimental effects on cognition of chronic as for short-term hyperglycaemia. Chronic hyperglycaemia can be identified in tests of fasting plasma glucose, glucose tolerance or glycosylated haemoglobin (HbA1c). The latter is considered the ‘gold-standard’ (Lamport, et al., 2009), because it reflects the glucose which haemoglobin cells have been exposed to over their cell cycle of two to three months.

A number of observational studies of general middle-aged to older populations have linked higher blood glucose levels to lower level of cognitive ability short of dementia (Cartwright, et al., 2012; Tournoy et al., 2010). In one non-diabetic old-old cohort, increases in HbA1c during mean 32-month follow-up were also related to concurrent cognitive decline (Ravona-Springer et al., 2012). Individuals with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) are exposed to chronically (for IFG) or periodically (for IGT) elevated blood glucose levels, and both of these pre-diabetic states have also been related to lower level of late-life cognitive function short of dementia (Di Bonito et al., 2007; Yaffe, Blackwell, et al., 2004). A systematic review of studies carried out in adults of any age supports these findings. Prospective analyses were less consistent. IGT appears to predict cognitive decline only when subjects remain in a pre-diabetic state in the long-term (Lamport, et al., 2009), and since publication of the review, an analysis of a Scottish cohort found that higher baseline fasting glucose predicted a trend for *decelerated* annual decline in memory (Euser, et al., 2010).

The way in which hyperglycaemia may influence cognitive function may involve associations with micro- and macrovascular disease and with endothelial dysfunction (Brownlee, 2001; Lim, MacFayden, Bakris, & Lip, 2006). Basis for this may be an increased formation of advanced glycation endproducts (AGE), which are produced intra- and extracellularly in an irreversible reaction of glucose and other reducing sugars with protein amino groups (Brownlee, 2001; Semba, Nicklett, & Ferrucci, 2010). In interaction with cell surface receptors (RAGE), AGE increase levels of inflammation and oxidative stress on vascular walls (Méndez, Xie, Aguilar-Hernández, & Méndez-Valenzuela, 2010). Their role as a risk factor for cognitive decline was shown in associations of higher AGE with an increased rate of cognitive decline short of dementia in a prospective study of healthy older adults and of people with diabetes (Yaffe et al., 2011). AGE have also been identified within neurofibrillary tangles and senile plaques, which has led to the suggestion of a potential role in the pathogenesis of Alzheimer's Disease (Ryan & Geckle, 2000).

Chronic hyperglycaemia and cognition in people with diabetes

In younger adults with type 1 diabetes (Northam & Lin, 2010) as well as in type 2 diabetes (Cukierman-Yaffe et al., 2009; de Wet, Levitt, & Tipping, 2007; Greenwood, et al., 2003; Munshi et al., 2006), a number of observational studies have found associations of higher chronically elevated glucose levels with poorer level of cognitive function, typically with medium to large effect size. For instance, in one study of patients with type 2 diabetes, HbA1c ≥ 7 was associated with a significant odds ratio of 4.9 for presence of reduced executive function, which was identified using a screening test (de Wet, et al., 2007).

Observational evidence is supplemented by intervention studies manipulating the level of glycaemic control. In the Action to Control Cardiovascular Risk in Diabetes-Memory in Diabetes (ACCORD-MIND) RCT study of almost 3000 older patients with type 2 diabetes, a statistically non-significant trend for decelerated 20-month decline in processing speed (but no other age-sensitive cognitive domains) was observed for an intensive treatment group compared with a conventional treatment group. The former also showed significantly slower rates of decline in total brain volume (-13% versus -18%) during 40-month follow-up (Launer et al., 2011). A smaller RCT of patients with diabetes suggested an important role of post-prandial glucose levels in particular. Whereas both treatment groups achieved a reduction in HbA1c, only one achieved better post-prandial glucose control, and this was also the only group which did not cognitively decline over the one-year duration of the trial (Abbatecola et al., 2006).

Overall, and consistent with some evidence which suggests that at least for people aged below 70 years, diabetes may only have a small effect on cognition when good glycaemic control is maintained (Messier, 2005), associations between raised blood glucose levels and poorer cognitive ability appear relatively well-established in general and in diabetic populations. Yet, the rare prospective observational analyses and intervention studies have produced inconsistent findings, resulting in continued debate on the possible causal role of hyperglycaemia in age-related cognitive decline.

2.2 Vascular risk factors

2.2.1 Endothelial dysfunction and hypertension

Normal endothelial function is important for the adaptation of blood vessels to changes in blood flow via the release of vasodilatory (e.g., nitric oxide; NO) and vasoconstrictive molecules (e.g., endothelin-1) in their endothelial surface (Sundell, 2005). Endothelial function may be measured using soluble plasma biomarkers (Markus et al., 2005) or the change in brachial artery diameter following induction of mechanical ischemia (R. A. Cohen et al., 2009). Endothelial dysfunction may have a role to play in the development of atherosclerosis, in which lipids, fatty streaks and atherosclerotic plaques develop in the arterial endothelium. Arteries ultimately stiffen and calcify, narrowing the arterial lumen and thickening the intima layer (Robertson, Fowkes, & Price, 2012) and to compensate for a reduction in blood flow caused by atherosclerosis, blood pressure increases, sometimes to a level defined as hypertension (systolic pressure >140mmHg, diastolic pressure >90mmHg (World Health Organization, 2011)). Factors associated with obesity and metabolic derangement can contribute to this process (Sundell, 2005). Featuring the largest small-vessel and microcirculatory endothelial surface of the body (Román, 2005), the brain is vulnerable to these processes. Atherosclerosis, hypertension and endothelial dysfunction have been linked to white matter disease (R. A. Cohen, et al., 2009; Hoth et al., 2007; Kearney-Schwartz et al., 2009; Markus, et al., 2005), silent cerebral infarcts (Dempsey, Vemuganti, Varhese, & Hermann, 2010), cerebral hypoperfusion and hypoxia (Skoog & Gustafson, 2006).

Endothelial dysfunction, hypertension and cognition in the general population

A small number of studies have examined endothelial dysfunction in relation to cognition. In two cohorts of middle-aged to older adults with cardiovascular disease, endothelial dysfunction measured by brachial response was associated with lower level of cognitive function short of dementia (R. A. Cohen, et al., 2009; Forman et al., 2008); one additionally reported inverse correlations with total brain volume (R. A. Cohen, et al., 2009).

Hypertension and cognition in the general population has been researched more extensively. Associations of the potential risk factor with reduced cognitive function were reported as early as the 1950s (Apter, Halstead, & Heimburger, 1951) and are now supported by findings from prospective investigations (Swan et al., 1998; Wilkie & Eisdorfer, 1971). In one study, each 10mmHg increase in midlife systolic blood pressure predicted a 5% increased risk of poorer cognitive function in later life (Launer, Masaki, Petrovitch, Foley, & Havlik, 1995). Despite some conflicting evidence (Glynn et al., 1999; Yu, Ryan, Schaie, Willis, & Kolanowski, 2009), a systematic review of 24 cross-sectional and prospective studies revealed consistent detrimental effects of small to medium effect size of hypertension on global cognitive function, memory, processing speed, executive function and attention, with similar results in the analysis of late-life hypertension and of mid-life hypertension (van den Berg, et al., 2009). Some studies also reported links of hypertension with presence of clinically diagnosed dementia (Hayden et al., 2006; Launer et al., 2000). Meta-analyses revealed odds ratios of 1.6 and even 4.8 for vascular dementia on the basis of prospective and cross-sectional studies, respectively (Sharp et al., 2011), although the risk of Alzheimer's Disease appears to be relatively unrelated to hypertension (Guan et al., 2011).

Hypertension and cognition in people with diabetes

Atherosclerosis, arterial wall stiffening, hypertension and endothelial dysfunction are all common in people with type 2 diabetes (D'Souza et al., 2009; Jensen-Urstad, Reichard, Rosfors, Lindblad, & Jensen-Urstad, 1996; Stehouwer, Henry, & Ferreira, 2008). The limited evidence on hypertension and cognition in such patients suggests similar associations as in the general population. In a cross-sectional analysis of the Framingham Study, patients with diabetes and hypertension were at 68% increased risk of poor visual memory and at 103% increased risk of low scores on a composite measure of cognitive function (relative to the sample) compared with normotensive patients (P. K. Elias et al., 1997). A Russian study of middle-aged women with type 2 diabetes also reported higher prevalence of 'abnormalities' in executive function in subjects with hypertension (Petrova, Prokopenko, Pronina, & Mozheyko, 2010), although the findings were limited by the relatively young age of the sample (mean age 56 years) and the unclear definition of cognitive outcomes. One cross-sectional study of type 2 diabetes patients, which treated cognition on as a continuous outcome, reported associations between hypertension and lower verbal memory, but statistical results were not reported (Mount, 2011). In a rare prospective investigation of 258 old-old dementia-free individuals, the presence of hypertension also exacerbated diabetes-related cognitive decline. Patients with diabetes declined by -0.29 standard deviations on the MMSE during two-year follow-up, whereas patients with co-morbid diabetes and hypertension declined by 0.42 standard deviations. Nineteen % of patients with diabetes suffered incident dementia compared with 23% of individuals with co-morbid diabetes and hypertension (Hassing, et al., 2004).

Antihypertensive therapy and cognition

Studies on the effects of antihypertensive treatment (e.g., calcium channel blockers, diuretics, beta-blockers, angiotensin-converting enzyme inhibitors or angiotensin II-receptor blockers) could potentially help resolve the question of whether hypertension has a causal role in old-age cognitive decline. However, to date, a mixture of RCTs and observational studies (which are much more likely to suffer from confounding), have produced mixed results, with all systematic reviews and meta-analyses (Chang-Quan et al., 2011; Feigin, Ratnasabapathy, & Anderson, 2005; Qiu, Winblad, & Fratiglioni, 2005) except one (K. Shah et al., 2009), as well as a recent review by the Cochrane group on evidence restricted to RCTs (McGuiness, Craig, Bullock, & Passmore, 2009), showing that the totality of evidence is inconclusive. One meta-analysis notes that should beneficial treatment effects on cognition indeed exist, these may be restricted to reductions in risk of vascular dementia (Chang-Quan, et al., 2011), consistent with treatment-induced reduction in risk of cerebral infarction (PROGRESS Collaborative Group, 2001). Since these meta-analyses were carried out, two prospective investigations have reported beneficial treatment effects on performance on the MMSE and detailed cognitive tests, but these were relatively small ($n \leq 100$) intervention studies with short follow-up (Jaiswal, Bhavsar, Jaykaran, & Kantharia, 2010; Kennelly et al., 2011). One observational study revealed no consistent relationship between antihypertensive treatment and six-year risk of dementia (P. Schneider et al., 2011).

Investigations of antihypertensive treatment and cognition in populations with diabetes are rare. One US study of hospital records on almost 380 000 older diabetes patients found that antihypertensive treatment decreased the two-year risk of dementia diagnosis by between 4% and 24% depending on the specific treatment (M. L. Johnson et al., 2012).

Importantly, any effects of antihypertensive medications on cognition may be unrelated to a reduction in blood pressure itself, rather reflecting their effect on biological pathways, such as the protective effect of calcium channel blockers on calcium build-up in neurons (Stampfer, 2006). Conversely, one report of improved cognitive test performance following non-pharmaceutical treatment of hypertension (Vanderploeg, Goldman, & Kleinman, 1987) would support direct effects of blood pressure reduction on cognition.

2.2.2 Inflammation

Systemic inflammation is associated with a wide range of morbidities and behaviours, including diabetes, hypertension, physical exertion, sleep disturbances, uremia, chronic fatigue, depression, adiposity, over-reactivity of the immune system, smoking, vascular injury, use of hormone replacement therapy and alcohol intake (Kushner, 2001; Schmidt et al., 2002). Inflammation is an adaptive mechanism, which becomes problematic when it persists long-term, and occurs as 'inflammaging' during normal ageing. The body is increasingly burdened by chronic low-grade inflammation caused by infection, tissue damage or a deterioration of repair mechanisms (Singh et al., 2008).

Observational and intervention studies in the general population on the effects of anti-inflammatory or anti-oxidant therapy have linked a reduction in systemic inflammation to improved cognitive outcome. For example, reviews of the literature on observational analyses have concluded that the evidence suggests a reduced risk of dementia following the long-term use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) (McGeer, Schulzer, & McGeer, 1996) or high dietary antioxidant intake (Ancelin, Christen, & Ritchie, 2007). For instance, a higher antioxidant intake was associated with a higher level of cognitive function at age 70 in a Scottish birth cohort. The effect sizes of the findings were small (McNeill et al., 2011), but intervention studies lend support to significant contributions by inflammation. In one study of younger adults, bacterial infection caused both increased levels of inflammatory cytokines and declines in memory (C. Holmes, 2013), and some RCTs have revealed beneficial effects of antioxidant treatment on cognitive function in animals, and in humans with or without dementia diagnosis, although a review of the literature notes that this type of evidence overall appears to be inconclusive (Ancelin, et al., 2007).

The way in which inflammation may impact on cognitive function involves the initiation of Alzheimer's Disease-typical pathology (Akiyama et al., 2000; Giunta et al., 2008). In one study inflammation even predicted whether or not such brain pathology manifests in symptoms of dementia (Arosio et al., 2011). Inflammation further appears to initiate neurodegeneration (Maier & Watkins, 1998; cited in (Marsland et al., 2006)), the activation of astrocytes and microglia (Shalev, et al., 2009), and has a bidirectional relationship with vascular disease. Inflammatory proteins have been shown to contribute to atherogenesis and endothelial dysfunction (Kuo et al., 2005; Libby, et al., 2002), and damage to the vessel wall appears to lead to a pro-inflammatory response (Nash, 2005).

Circulating markers of inflammation include c-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen and tumor necrosis factor alpha (TNF-α), all of which typically correlate strongly (Brunsgaard et al., 1999; Yudkin, Stehouwer, Emeis, & Coppack, 1999). The observational evidence from general older and diabetic populations on associations of each marker with cognition is reviewed in the sections below.

C-reactive protein (CRP)

The pentraxin protein CRP is produced in the liver and in adipose tissue. It is a 'first-class' acute phase reactant extremely sensitive to inflammation (Teunissen et al.,

2003) and is regulated by IL-6 (Kuo, et al., 2005). Due to its ability to initiate cell lysis and phagocytosis of pathogens and cell debris, CRP is extremely important to the immune system (Schmidt, et al., 2002), but chronically raised levels have been linked to poor health outcomes (Harris et al., 1999).

CRP and cognition in the general population

Despite high intra-individual variability in CRP (de Maat et al., 1996), single-measurement CRP has been associated with poorer level of cognitive function short of dementia assessed on detailed cognitive tests or screening instruments in several cohorts of middle-aged to older individuals (Dik et al., 2005; Hogue et al., 2006; Luciano, Marioni, Gow, Starr, & Deary, 2009; Marioni, Deary, et al., 2010; Marioni et al., 2009; Ravaglia et al., 2005; Schram et al., 2007), but findings are not entirely consistent. In one recent study of old-old adults, a composite measure of CRP and TNF-α was associated with lower visuospatial ability, but was unrelated to other domains or to global cognitive function (Arfanakis et al., 2013). Observational studies assessing change in cognitive function have also produced mixed results. Cross-sectional analyses of one Scottish cohort (Aspirin for Asymptomatic Atherosclerosis Trial, AAA) and one Dutch cohort found that associations between CRP and cognitive function persisted when estimates of peak pre-morbid ability were controlled for (Marioni, Deary, Murray, Lowe, et al., 2010; Teunissen, et al., 2003). This suggests steeper estimated lifetime decline in individuals with higher late-life CRP. However, associations between CRP and level of cognitive function did not survive or substantially attenuated following adjustment for (estimated) pre-morbid ability or for its proxy education in another Dutch cohort (Dik, et al., 2005) and in three further Scottish cohorts (including one with data available on IQ at age 11) (Luciano, et al., 2009; Marioni, Deary, Murray, Lowe, et al., 2010). This indicates a potential confounding role of peak pre-morbid ability in any cross-sectional links between higher inflammation and lower late-life cognitive function.

In terms of prospective analyses of late-life cognitive change, baseline CRP predicted the rate of five-year decline short of dementia on some cognitive domains in the AAA trial (Marioni, et al., 2009). Similar findings were reported for another similarly large cohort with two- to five-year follow-up (Yaffe, Kanaya, et al., 2004;

Yaffe et al., 2003). However, effect sizes in these studies appeared to be small, and other prospective studies failed to report similar findings (Dik, et al., 2005; Schram, et al., 2007; Teunissen, et al., 2003). Oddly, in one study, CRP was unrelated to decline in several age-sensitive cognitive domains, but predicted steeper decline on the Mill-Hill Vocabulary Scale - an estimate of pre-morbid ability which is typically relatively immune to decline (Gimeno, Marmot, & Singh-Manoux, 2008).

CRP has also been linked to risk of dementia in several large-scale prospective analyses with two- to 25-year follow-ups (Schmidt, et al., 2002; G. Xu, Zhou, Zhu, Fan, & Liu, 2009; Yaffe, Kanaya, et al., 2004). For instance, in the Honolulu-Asia Aging Study, baseline CRP in the upper three (versus the lowest) quartiles was linked to a three-fold increased risk of dementia 25 years later (Schmidt, et al., 2002). One investigation failed to establish such an association (Licastro et al., 2000). Importantly, in all studies which report significant findings, these were restricted to non-Alzheimer's Disease types of dementia, in line with a number of cross-sectional analyses, which have consistently revealed equivalent CRP levels in Alzheimer's Disease patients and controls (Engelhart et al., 2004; Giometto, Argentiero, Sanson, Ongaro, & Tavolato, 1988; Schmidt, et al., 2002; G. Xu, et al., 2009).

The interpretation of evidence on CRP and cognition is complicated by heterogeneity in sample characteristics. For instance, in studies of older adults without dementia, mean CRP levels reportedly ranged between 5.7 mg/l and 12.5 mg/l (Hogue, et al., 2006; Teunissen, et al., 2003). Acknowledging this heterogeneity, two reviews of the literature note that meta-analysis was not possible, but that evidence overall suggests that higher CRP predicts steeper late-life cognitive decline as well as increased risk of dementia (Kuo, et al., 2005; Schram, et al., 2007).

CRP and cognition in people with diabetes

Type 2 diabetes is characterised by systemic inflammation, and some evidence suggests that elevated CRP in particular may increase the risk of future diabetes (Freeman et al., 2002). Yet, studies on CRP and cognition in this type of patient are rare. In a cross-sectional analysis of 115 type 2 diabetes patients undergoing surgery, higher pre-operative CRP was associated with poorer cognitive function short of dementia one day after surgery (Heyer, et al., 2013). Patients with reduced cognitive function short of dementia also had higher mean CRP compared with higher functioning patients in a similarly small study of hospitalised type 2 diabetes patients, with medium effect size of the association (G. Chen et al., 2011). In a cross-sectional analysis of the only population-based study investigating CRP in diabetes to date (the Edinburgh Type 2 Diabetes Study), CRP levels were associated with lower estimated peak pre-morbid ability and lower reasoning, with an additional trend short of statistical significance for lower global ability. However, findings did not survive adjustment for the estimate of peak pre-morbid ability, suggesting that the findings were driven by peak pre-morbid ability. Yet, in support of potentially causal CRP-cognition associations, genetic variants of a gene associated with raised levels of CRP were linked to lower level of ability in several cognitive domains in the same study (Marioni, Deary, Murray, Lowe, et al., 2010).

Interleukin-6 (IL-6)

IL-6 is secreted by monocytes, astrocytes, endothelial cells and neurons (amongst others) in response to TNF-α and endotoxin (Barkhudaryan & Dunn, 1999). Levels increase with volume of subcutaneous adipose tissue (Yudkin, et al., 1999) and have been associated with poor health outcomes (Harris, et al., 1999).

IL-6 and cognition in the general population

A number of cross-sectional studies have linked higher levels of circulating IL-6 to lower cognitive function short of dementia in general middle-aged or older cohorts (Gimeno, et al., 2008; Marsland, et al., 2006; Rafnsson et al., 2007; Weaver et al., 2002; Wikby et al., 2005; Wright et al., 2006a) and in patients with multiple sclerosis (Pantanella et al., 2010). IL-6 may also be raised in individuals with diagnosed dementia (Wada-Isoe, Wakutani, Urakami, & Nakashima, 2004), and has been related to functional status in dementia (Zuliani et al., 2007).

With respect to cognitive change, higher IL-6 has been associated with steeper estimated lifetime cognitive decline in cross-sectional analyses with adjustment for estimates of peak pre-morbid ability (Marsland, et al., 2006; Rafnsson, Deary, Smith, Whiteman, Rumley, et al., 2007). In prospective investigations, IL-6 in the highest tertile (versus the remaining two tertiles or versus the lowest tertile) predicted a 34% to 100% increased risk of 'steep' cognitive decline short of dementia (e.g., >5 point-decline on MMSE) during 2.5 year, seven-year (Weaver, et al., 2002) or two-year follow-ups (Yaffe, et al., 2003). In the Edinburgh Artery Study, higher baseline IL-6 was associated with a steeper four-year decline in later life, although findings did not survive adjustment for cardiovascular disease, vascular risk factors, depression and diabetes status (Rafnsson, Deary, Smith, Whiteman, Rumley, et al., 2007). Another British study also failed to establish associations of IL-6 with cognitive decline short of dementia. An exception was the Mill-Hill Vocabulary Scale, which may be used to estimate peak pre-morbid ability. For this test, a decline was predicted by higher baseline IL-6 (as had also been found for CRP in the same study) (Gimeno, et al., 2008). As for CRP, studies on IL-6 are heterogeneous, with mean IL-6 levels ranging between 1.6 pg/ml (Wright et al., 2006b) and 4.6 pg/ml (Weaver, et al., 2002), and the literature on IL-6 and cognition in the general population has not been meta-analysed to date.

IL-6 and cognition in people with diabetes

Consistent with increased systemic inflammation in diabetes, endothelial expression of IL-6 appears to be increased in patients with diabetes (Hartge, Unger, & Kintscher, 2007). The limited evidence points to significant associations of IL-6 with cognition in patients with type 2 diabetes as in the general population. In the Edinburgh Type 2 Diabetes Study, higher IL-6 was linked to lower level of cognitive function short of dementia. Specifically, each two-fold increase in IL-6 was associated with 0.17 lower scores on a global ability factor. The finding survived adjustment for an estimate of peak pre-morbid ability, suggesting steeper estimated lifetime decline in individuals with higher late-life IL-6 (Marioni, Strachan, et al., 2010).

Finally, in support of an important role of IL-6 in cognition, knockout of the IL-6 gene appears to improve learning ability in rodents (Braida et al., 2004). In the general human population, genetic variants associated with levels of IL-6 may also predict the level of cognitive function short of dementia in older age (Sasayama et al., 2011), as well as the age at onset of Alzheimer's Disease (Papassotiropolous et al., 1999), although the evidence overall is inconsistent (Baune et al., 2008; Eriksson et al., 2011; Mansoori et al., 2010; Marioni, Deary, Murray, Fowkes, & Price, 2010).

Fibrinogen

The acute-phase cytokine fibrinogen is produced in the liver (Schultz & Arnold, 1990), and circulating levels are affected by sex, ethnicity, socioeconomic status and vascular risk factors (Fibrinogen Studies Collaboration, 2007).

Fibrinogen and cognition in the general population

Several cross-sectional analyses (Wada et al., 2011), including three of Scottish middle-aged to older cohorts (Luciano, et al., 2009; Marioni, et al., 2009; Rafnsson, Deary, Smith, Whiteman, Rumley, et al., 2007), have associated higher fibrinogen with lower late-life cognitive function short of dementia in the general population, although others have failed to establish such findings (Elwood, Pickering, & Gallacher, 2001; J. Huang et al., 2011). In one Japanese study of older adults, higher fibrinogen was also associated with a higher grade of white matter lesion with small to medium effect size (r=0.19) (Wada, et al., 2011). In the Edinburgh Artery Study which adjusted for vocabulary-based estimated peak pre-morbid ability in cross-sectional analyses and also had a prospective follow-up four years after baseline, higher fibrinogen was associated an increased rate of estimated lifetime decline in reasoning and with steeper actual late-life decline in verbal memory, although it was unrelated to decline in global ability (Rafnsson, Deary, Smith, Whiteman, Rumley, et al., 2007). One prospective Scottish study on actual lifetime cognitive change between childhood and older age, found that associations between higher late-life levels of fibrinogen and steeper lifetime cognitive decline were restricted to a measure of reaction time (Luciano, et al., 2009). Another prospective investigation carried out in middle-age to older adults found fibrinogen to be unrelated to five-year decline on a number of cognitive domains, with the exception of a test of executive function (Marioni, et al., 2009). Finally, fibrinogen has also been linked to future risk of dementia of any type, vascular dementia and Alzheimer's Disease in several prospective investigations (van Oijen, Witteman, Hofman, Koudstaal, & Breteler, 2005; G. Xu, Zhang, Zhang, Fan, & Liu, 2008). For instance, in one study each standard deviation increase in fibrinogen increased the risk of dementia by 26% during six years (van Oijen, et al., 2005).

Fibrinogen and cognition in people with diabetes

Levels of fibrinogen are raised in type 2 diabetes (Dunn & Ariëns, 2004), but the investigation of their relationship with cognition has been neglected in this group of patients. In the Edinburgh Type 2 Diabetes Study, higher fibrinogen was associated with lower global ability. Each unit increase in fibrinogen was associated with a decrease by 0.09 on a global ability factor (Marioni et al., 2011). Genetic variants associated with fibrinogen were further significantly related to late-life level of cognitive function (although the association between the genetic variant and fibrinogen levels were relatively weak in this cohort) (Marioni, et al., 2011). The latter finding was contrasted by investigations of fibrinogen polymorphism and cognition in the general population, which to date has been limited to analyses of three Scottish cohorts and have produced inconsistent results (Marioni, Deary, Murray, Lowe, et al., 2010; Marioni, et al., 2011).

Tumor necrosis factor alpha (TNF-α)

TNF-α is the 'master cytokine' which initiates the inflammatory cascade (Clark, et al., 2012) and has been linked to chemical processes in the central nervous system. Beta amyloid deposition in AD initiates TNF-α production (Medeiros et al., 2010), and the cytokine acts on afferent fibres in the spinal cord and interacts with neurotransmitters, including glutamate (G. M. Holmes, Hebert, Rogers, & Hermann, 2004).

TNF-α and cognition in the general population

The evidence on associations of TNF-α with cognition is mixed and with exception of a single study showing that serum levels were raised in people with cognitive impairment short of dementia as well as in patients with Alzheimer's disease (Àlvarez, Cacabelos, Sanepro, Garcìa-Fantini, & Aleixandre, 2007), the literature to date has focused on dementia rather than on the spectrum of late-life cognitive function and decline. A number of relatively small cross-sectional studies reported raised plasma levels in patients with Alzheimer's Disease relative to controls with a medium effect size of unadjusted associations (Brunsgaard, et al., 1999; Fillit et al., 1991). In direct contrast, two further investigations set in the Netherlands and Japan found equivalent levels of TNF-α in patients with Alzheimer's Disease, in those with vascular dementia and in controls (Engelborghs et al., 1999; Yasutake, Kuroda, Yanagawa, Okamura, & Yoneda, 2006). Another study even reported lower levels in Alzheimer's Disease and multi-infarct dementia than in age-matched controls (Cacabelos, Alvarez, Franco-Maside, Fernández-Novoa, & Caamaño, 1994), although the latter finding could reflect survival bias of patients with lower TNF-α. A rare prospective investigation reported a higher number of TNF receptors in plasma and cerebrospinal fluid of individuals who went on to develop dementia compared with a group who remained cognitively stable during four to six years. Effect sizes of unadjusted group comparisons were of medium size (Cohen's d=0.33 to d=0.54; depending on the specific TNF receptor under investigation) (Buchhave et al., 2010; J. Cohen, 1992).

In support of TNF-α associations with cognition, genetic variants coding for TNF-α have been found to predict the level of late-life cognitive function short of dementia (Baune, et al., 2008), as well as the presence or risk of dementia (Albani et al., 2012; Brunsgaard et al., 2004; Fung et al., 2005), although this type of evidence overall is also inconsistent (Pantanella, et al., 2010). Finally, causal effects of TNF-α on cognition are supported by beneficial effects of anti-TNF therapy on improved cognitive function in an intervention study of older adults with rheumatoid arthritis and by reduced beta amyloid deposition following such therapy in rodents (Y. M. Chen, Chen, Lan, & Chen, 2010; Medeiros, et al., 2010).

2.2.3 Smoking

Smoking is a vascular risk factor with complex relationships to other risk factors. Smokers tend to come from lower socioeconomic backgrounds (Hiscock, 2007), have greater abdominal fat mass and diabetes risk, but also have lower BMI compared with non-smokers (Tonstad, 2009; Wild & Byrne, 2006). Smoking cessation is linked to weight gain (Wild & Byrne, 2006). Research into smoking and cognition is complicated, because data is particularly affected by survival bias, selective attrition and recall bias. Overall, however, the literature has revealed relatively consistent findings of negative associations between smoking and cognition.

Smoking and cognition in the general population

Cross-sectional evidence from cohorts from the general population have revealed links between smoking and lower late-life cognitive function short of dementia (Corley, Gow, Starr, & Deary, 2012; Stewart, Deary, Fowkes, & Price, 2006). Such evidence may be restricted by observations that estimated or actually measured childhood ability predicts later smoking (Corley, et al., 2012; Richards, Jarvis, Thompson, & Wadsworth, 2003; Stewart, et al., 2006), but associations of smoking with lower late-life cognitive function in the Lothian Birth Cohort 1936 survived adjustment for IQ at age 11 showing that findings were independent of potential confounding by pre-morbid ability (Corley, et al., 2012).

Instead, associations of smoking with poorer cognitive outcome are likely to involve a degree of causality. Although nicotine itself appears to facilitate neurotransmission in the cholinergic system and has neuroprotective and anti-oxidant properties (Swan & Lessov-Schlaggar, 2007), a total of 4700 compounds of smoke have toxic properties. Due to carboxyhemoglobin, smoking contributes to deficiencies in oxygen supply. It increases the vascular burden of the body through an increase in platelet aggregability and decreases HDL-cholesterol levels, initiating atherosclerosis, and contributes to reduced blood-brain barrier function, to oxidative stress, inflammation and reduced anti-oxidant levels (R. S. Shah & Cole, 2010; Swan & Lessov-Schlaggar, 2007).

In support of a causal relationship, prospective investigations have also revealed significant associations. For instance, in one English study of 3000 middle-aged adults, heavy smoking at age 53 (>20 cigarettes/day) was linked to a mean decline in memory since age 43 which was two words greater (4% of total words to be recalled) compared with non-smokers at age 53 (Richards, et al., 2003). A systematic review of prospective studies concluded that although having ever smoked does not appear to predispose individuals to steeper late-life cognitive decline short of dementia compared to people who have never smoked, current smokers are at increased risk of cognitive decline compared with former smokers and those who never smoked (Anstey, von Sanden, Salim, & O'Kearney, 2007). Although another systematic review established only a non-significant trend for the latter association (R. Peters et al., 2008), two more recent investigations support the evidence for current smokers (compared with former/ never smokers) experiencing steeper late-life cognitive decline (N. Collins, Sachs-Ericsson, Preacher, Sheffield, & Markides, 2009; Nooyens, van Gelder, & Verschuren, 2008). One recent study, which revealed a three-fold *lower* ten-year risk of reduced cognitive function short of dementia in current/ past smokers compared with people who had never smoked seems to be at odds with the remaining literature (C.-C. Wang et al., 2010).

Prevalence of smoking may be reduced in patients with diagnosed dementia (Lester, Lyubarova, Kirtani, Macina, & Kohen, 2011), but a number of prospective investigations have linked smoking to an increased risk of future dementia. One large US study reported linear dose-response relationships between the amount smoked (up to heavy smoking) and risk of Alzheimer's Disease over several decades of follow-up, as well as with density of neurotic plaques (Tyas et al., 2003). A meta-analysis of prospective studies overall reported 1.8-fold increased risks for both Alzheimer's Disease and vascular dementia in current smokers versus former and never smokers. Risk of Alzheimer's Disease was even increased for current smokers compared with former smokers, suggesting a potential for the brain to 'recover' from damage (Anstey, et al., 2007). Since the systematic review was completed, further prospective investigations of smoking and risk of dementia have supported this evidence (Rusanen, Kivipelto, Quesenberry, Zhou, & Whitmer, 2011; Rusanen et al., 2010).

Smoking and cognition in people with type 2 diabetes

Smoking appears to be less prevalent in patients with type 2 diabetes compared with the general population (Schipf et al., 2009). Given that smokers are at increased risk of diabetes (Tonstad, 2009), this suggests that people may be likely to quit following diagnosis with diabetes. To date, studies have neglected the investigation of smoking and cognition in populations with diabetes. One early Dutch study of 489 men from a general older population found that individuals with cardiovascular disease and/or diabetes declined by 1.3 points on the MMSE (maximum score 30) during three years, compared with a 0.2 point improvement in people free of both of these risk factors (Launer, Feskens, Kalmijn, & Kromhout, 1996). However, because cardiovascular disease and diabetes were grouped together, the specific role of diabetes in this association is unclear. In another study exclusively of older patients with type 2 diabetes, smokers were less efficient in diabetes self-management compared with non-smokers, which led the authors to suggest poorer cognitive function in the group of smokers (Gucciardi, Mathew, Demelo, & Bondy, 2011).

Despite the overall lack of evidence, the findings for associations between smoking and poor cognitive outcome from the general population may well extend to people with diabetes. Because people with type 2 diabetes are less likely to smoke and because recent decreases in prevalence of smoking (R. S. Shah & Cole, 2010) contrasts the increasing prevalence of diabetes-associated cognitive dysfunction, smoking presumably does not account for the increased risk of poor cognitive outcome in diabetes.

2.3 Microvascular disease

2.3.1 Renal microvascular disease

Microvascular disease, or disease of small blood vessels, is often symptomless but may affect renal and retinal function. Renal microvascular dysfunction is caused by glomerular and tubular endothelial damage, and becomes evident as microalbuminuria (measured in the albumin/creatinine ratio) (Weiner et al., 2009). Microalbuminuria is also associated with clinical and subclinical macrovascular disease (H. Kramer et al., 2004; Yuyun et al., 2004). In the general population and in patients with macrovascular disease, microalbuminuria or low serum albumin levels have been associated with poorer level of cognitive function short of dementia (Kuo, Lin, & Yu, 2007; Llewellyn, Langa, Friedland, & Lang, 2010; Mizrahi, Blumstein, Arad, & Adunsky, 2008; Ng, Feng, Niti, & Yap, 2008). In unadjusted analyses of one study, individuals in the lowest tertile of albumin were also at almost two-fold increased risk of declines of ≥2 points on the MMSE during two year follow-up (Ng, Niti, Feng, Kua, & Yap, 2009).

Although microalbuminuria is present in around 20% of type 2 diabetes patients (Macisaac & Jerums, 2011), studies on this topic are rarely carried out in people with diabetes. In an analysis of over 28 000 patients with any type of diabetes (37%) and/or vascular disease, baseline microalbuminura was associated with a lower level of cognitive function short of dementia at baseline, and with a 22% increased risk of ≥3 point declines on the MMSE over five years (Barzilay et al., 2011). Overall, this suggests a similar association between renal microvascular disease and cognition in diabetic subjects as in the general population.

2.3.2 Retinopathy

Retinopathy is characterised by basement membrane thickening and loss of pericytes essential for retinal microvessel stability (Durham & Herman, 2011), and has been associated with metabolic derangements, including poor glucose control (Aso, Inukai, Tayama, & Takemura, 2000; WHO, 2011), dislipidemia and hypertension (Serlin, et al., 2011). Around 80% of insulin-treated patients with type 2 diabetes experience diabetic retinopathy (DR) within 20 to 25 years of disease onset (Durham & Herman, 2011), and DR accounts for around 5% of blindness globally (Chaturvedi, 2007).

Due to homology between retinal and cerebrovascular cells, the state of retinal small vessels closely mirrors the state of the microvasculature of the brain (Patton et al., 2005). This is reflected in associations between retinopathy and increased white matter hyperintensities (Ferguson et al., 2003), reduced grey matter volume (Musen et al., 2006) and increased number of silent brain infarcts (Kwon et al., 2007). In the general population, retinopathy has been associated with lower cognitive function short of dementia (M. L. Baker et al., 2007; Wong et al., 2002) and with presence of dementia (Devos et al., 2005).

Consistent with evidence from the general population, a cross-sectional analysis of the Edinburgh Type 2 Diabetes Study associated presence and severity of retinopathy with a lower level of cognitive function short of dementia in type 2 diabetes patients, with a small effect size of associations following multivariable adjustment (Ding et al., 2010). In an analysis of younger patients with type 1 diabetes, retinopathy was also associated with lower performance on a number of cognitive tests with moderate to large effect size. Additionally, 33% of individuals with retinopathy had evidence of white matter lesions of the basal ganglia, compared with only 4% of a retinopathy-free group (Ferguson, et al., 2003). Prospective analyses support these findings. One study of young to middle-aged type 1 diabetes patients reported that the development of DR over seven years was linked to steeper concurrent cognitive decline, although baseline DR was only weakly related to subsequent cognitive decline (Ryan, Geckle, & Orchard, 2003). A recent systematic review established a three-fold increased risk of poor cognitive outcome including dementia in patients with DR compared with those without (Crosby-Nwaobi, Sivaprasad, & Forbes, 2011), supporting results from a previous systematic review (Ding et al., 2008). Yet, more recently, evidence on associations between retinopathy and cognition from populations with diabetes and the general population has been mixed (de Bresser et al., 2010; Fergenbaum et al., 2010; Qiu et al., 2010).

2.4 Glucocorticoids

Cortisol is a glucocorticoid secreted from the adrenal glands and has receptors in a range of organs. In the brain, receptors are most abundant in the hippocampus and cortex (McEwen & Seeman, 1999). Cortisol levels follow a diurnal slope and are affected by the hypothalamus-pituitary-adrenal (HPA) axis, which functions according to mood and sensual stimulation (Björntorp & Rosmond, 2000). In the short-term, cortisol provides energy and increases survival (Porter & Landfield, 1998), but chronically raised levels due to disease (e.g., Cushing’s syndrome), stress, advanced age or obesity (Liu & Mori, 1999) are maladaptive.

2.4.1 Glucocorticoids and cognition in the general population

Cross-sectional analyses have linked high cortisol to ‘disorientation’ in stroke patients (Marklund, Peltonen, Nilsson, & Olsson, 2004), to lower prefrontal cortex and left hippocampal volumes in patients with psychiatric illness (Carrion, Weems, Richert, Hoffman, & Reiss, 2010; Mondelli et al., 2010), and to lower level of cognitive function short of dementia in the general middle-aged to older population (Beluche, Carrière, Ritchie, & Ancelin, 2010; Franz et al., 2011; Kilander, et al., 1997; Lara et al., 2013). Diurnal cortisol slopes may also be steeper in middle-aged individuals with higher cognitive function relative to those with lower cognitive function (Stawski et al., 2011). Although null results on cortisol and level of cognitive function short of dementia have occasionally also been reported (Schrijvers et al., 2011), one study reported higher cortisol in individuals with diagnosed dementia compared with dementia-free individuals (Lara, et al., 2013)

In addition to potential effects of cortisol on neuronal vulnerability (Porter & Landfield, 1998) and beta amyloid deposition (L. D. Baker et al., 2012), cross-sectional associations may be driven by associations of midlife cognitive ability with late-life cortisol levels (Franz, et al., 2011). However, several prospective investigations report similar evidence. In a French study, a flatter diurnal slope as well as higher morning cortisol predicted steeper four-year cognitive decline short of dementia, with odds ratios for scoring in the highest tertile of cognitive change scores ranging from 4.1 to 7.7 (Beluche, et al., 2010). Individuals with high or increasing cortisol levels over five to six-year follow-up also had lower cognitive function short of dementia, as well as 14% lower hippocampal volume at follow-up in a small Canadian study (Lupien et al., 1998). Yet, another prospective study of a relatively large cohort of older individuals from the general population found that baseline cortisol levels were unrelated to seven-year cognitive decline, or to the risk of dementia diagnosis during follow-up (Schrijvers, et al., 2011). Finally, higher baseline cortisol has been found to predict steeper cognitive decline in patients with diagnosed Alzheimer's Disease (Csernansky et al., 2006; C. W. Huang, Lui, Chang, Wang, & Chang, 2009).

2.4.2 Glucocorticoids and cognition in people with type 2 diabetes

Type 2 diabetes is associated with increased cortisol levels (Z. S. Lee et al., 1999), but to date only one study has assessed cortisol and cognition in this type of patient.

In a cross-sectional analysis of the Edinburgh Type 2 Diabetes Study, higher morning cortisol levels predicted steeper estimated lifetime decline in global ability, processing speed and working memory between estimated peak pre-morbid ability and late-life ability at the time of the clinic visit. Apart from a statistically non-significant trend for associations between higher cortisol and lower non-verbal memory, cortisol was entirely unrelated to current level of function at the clinic visit, however (R. M. Reynolds et al., 2010). In support of a link between cortisol and cognition in type 2 diabetes, one study reported that a blunted cortisol awakening response was linked to lower hippocampal volume in this type of patient. The unadjusted association was of large effect size (r=0.55) (Bruehl, et al., 2009).

Finally, the observational evidence from the general population and people with diabetes is supported by an analysis of effects of therapeutic intervention. In a study of 55 patients with major depressive disorder, the use of anti-depressant medication reduced cortisol levels and concurrently improved cognitive function (Hinkelmann et al., 2011). The literature on cortisol and cognition to date does not appear to have been meta-analysed or reviewed.

2.5 Apolipoprotein E

Cognitive ability is highly heritable, with genetic factors accounting for 30-80% of variance in cognitive test performance (Deary, et al., 2010). Amongst the genes currently known, the apolipoprotein E (APOE) gene linked to cholesterol levels, beta amyloid deposition (Stampfer, 2006) and neurofibrillary tangle formation (Peila, et al., 2002) appears to be the most important determinant of late-life cognitive outcome.

2.5.1 Apolipoprotein E and cognition in the general population

A recent meta-analysis of studies carried out in dementia-free individuals revealed that the presence of the APOEe4 allele (compared with the e2 or e3 alleles) has adverse effects on late-life performance in a range of cognitive domains, including global ability, with small effect size. Some evidence suggested that increasing age may exacerbate the strength of this association (Wisdom, Callahan, & Hawkins, 2011). Although genetic factors generally play less of a role in late-life cognitive decline compared with their role in the level of ability (Deary, 2012), the e4 allele has also been associated with late-life cognitive trajectories. In the Lothian Birth Cohort 1921, it predicted an annual decline on a test of verbal memory which was 0.5 points greater than was found for non-e4 carriers, and to a 0.3 point greater decline on a test of abstract reasoning (Schiepers et al., 2012).

The e4 allele is also the most important determinant of late-onset Alzheimer's Disease, alone accounting for around 20% of risk (Bookheimer & Burggren, 2009). One meta-analysis established odds ratios for Alzheimer's Disease of 3.7 for the e4 allele and 0.6 for the protective e2 allele relative to the e3 allele respectively (Williams, Plassman, Burke, Holsinger, & Benjamin, 2010).

2.5.2 Apolipoprotein E and cognition in people with diabetes

Presence of the e4 allele appears to exacerbate effects of diabetes on cognition. One cross-sectional analysis reports significant interactions between diabetes and e4 allele in their relationship with late-life level of cognitive function short of dementia (Dore, et al., 2009). These findings are supported by prospective cohorts of middle-aged to older adults which found that individuals with the e4 allele and diabetes (presumably of type 2) experienced steeper five- to seven-year cognitive decline compared with other combinations of APOE polymorphisms with diabetes status (Blair et al., 2005; Haan, Shemanski, Jagust, Manolio, & Kuller, 1999). Similar findings have been reported for risk of frank dementia. Several prospective studies have reported increased severity of Alzheimer's Disease-typical pathology and two to five-fold increased risk of diagnosis with Alzheimer's Disease in e4 carriers with type 2 diabetes compared with individuals with neither risk factors (Alafuzoff, Aho, Helisalmi, Mannermaa, & Soininen, 2009; Irie et al., 2008; Peila, et al., 2002; W. L. Xu, Qiu, Wahlin, Winblad, & Fratiglioni, 2004). These estimates of risks are all substantially larger than those reported in Section 1.7.3 for diabetes without consideration of APOE.

2.6 Psychosocial and lifestyle risk factors

2.6.1 Depression

In the general population, depression has been linked to presence of brain atrophy (Cole, Costafreda, McGuffin, & Fu, 2011) and reduced volumes of frontal gray matter, orbito-frontal cortex and amygdala (Egger, et al., 2008). Meta-analyses and reviews of the literature further revealed significant associations with a lower level of cognitive function (Harvey, Reichenberg, & Bowie, 2006), and with presence of dementia. For instance, dementia patients were found to be 80% more likely to be suffering from depression compared with cognitively healthy older indiviudals (C.-Q. Huang, Wang, Li, Xie, & Liu, 2011). A systematic review of prospective studies further revealed associations of depressive symptoms with steeper late-life cognitive decline which is short of dementia (Plassman, Williams, Burke, Holsinger, & Benjamin, 2010).

Depression and diabetes appear to have a bi-directional relationship (Lustman & Clouse, 2007). A meta-analysis estimated that depressed individuals are at 60% increased risk of type 2 diabetes, and that diagnosis with diabetes increases the risk of depression by 15% (Mezuk, Albrecht, Eaton, & Golden, 2008). Diabetes may cause depression through the initiation of certain biochemical and cerebrovascular changes (Bruce et al., 2006; Renn, Feliciano, & Segal, 2011), as well as ‘diabetes-associated distress’ stemming from perceived disability and psychological burden (Fisher et al., 2007). In support of importance of the latter, one study found that prevalence of depression was raised in individuals with diagnosed but not in people with undiagnosed type 2 diabetes (Nouwen et al., 2011).

The evidence from cross-sectional studies comparing diabetes patients with and without depressive symptoms or clinical depression is mixed (Trento et al., 2011; Watari et al., 2006). Additive detrimental effects are suggested in a prospective analysis of a large US cohort which found that co-morbidity of diabetes and depression was linked to a 100% increased risk of dementia over three to five-year follow-up when compared with presence of diabetes without depression (Katon et al., 2012).

2.6.2 Cognitive and social engagement

In line with the concept of ‘use it or lose it’, a number of studies of the general population have shown that cognitively stimulating or leisure activities may protect from age-related cognitive decline and may facilitate cognitive improvement (Leung et al., 2011; Marioni, et al., 2012; Wilson et al., 2003). Social engagement has also been linked to a higher level of late-life cognitive function (Barnes, Mendes de Leon, Wilson, Bienias, & Evans, 2004), to a reduced rate of cognitive decline short of dementia (Andrew & Rockwood, 2010; Barnes, et al., 2004; Béland, Zunzunegui, Alvarado, Otero, & del Ser, 2005) and in two studies to around 40% to 50% lower risk of future dementia over three years (Fabrigoule et al., 1995; Fratiglioni, Wang, Ericsson, Maytan, & Winblad, 2000). Yet, the evidence overall is mixed for both cognitive (Helzner, Scarmeas, Cosentino, Portet, & Stern, 2007; Wilson et al., 2000) and for social engagement (Hultsch, Hertzog, Small, & Dixon, 1999; Stoykova, Matharan, Dartigues, & Amieva, 2011), and reviews of the literature have come to inconsistent conclusions (Fratiglioni, Paillard-Borg, & Winblad, 2004; Williams, et al., 2010). One of the main issues in the examination of evidence on this topic appears to be the uncertain direction of associations. While it is plausible that cognitive and social engagement protects from brain ageing, people with lower pre-morbid ability or those already affected by early signs of cognitive declines may be less likely to engage in such activities.

2.6.3 Physical activity

Although a meta-analysis notes that the evidence for cross-sectional protective effects of physical activity on cognition overall is relatively weak (Etnier, Nowell, Landers, & Sibley, 2006), a review of prospective studies of humans and rodents has inked exercise to increased cerebral blood flow, changes in wave frequencies and amplitudes during cognitive processing, hippocampal neurogenesis, cell proliferation, cell survival (Hillman, Erickson, & Kramer, 2008), and in one study further to increased synaptic plasticity (A. Wu, Ying, & Gomez-Pinilla, 2008). Meta-analyses further reveal activity-associated reduction in risk of cognitive decline short of dementia (Sofi et al., 2011) and risk of dementia (Hamer & Chida, 2009) by 38% and 28%, respectively. Consequently, intervention programs in non-demented older adults may have beneficial effects on cognitive function, as was determined in a recent systematic review of twelve RCTs, eight of which reported significant findings (Tseng, Gau, & Lou, 2011).

In type 2 diabetes, higher physical activity has been associated with a higher level of late-life cognitive function (Colberg, et al., 2008; Devore, Kang, Okereke, & Grodstein, 2009) and in one study of older females was linked to reduced rate of cognitive decline. A mean difference in decline in a measure of global ability of 0.14 standard units was found between a high-activity compared with a low-activity group (Devore, Kang, et al., 2009). The sedentary lifestyle typical of type 2 diabetes (Morrato, Hill, Wyatt, Ghushchyan, & Sullivan, 2007) undoubtedly contributes to the increased risk of poor cognitive outcome in diabetes patients, but the hypothesis by one researcher that low physical activity is the main cause of diabetes-associated cognitive decline (Brito, 2009) appears controversial.

2.6.4 Nutrition

A number of reviews of the literature have shown that high intake of saturated fats (Solfrizzi et al., 2005), high cholesterol and low antioxidant intake (Kanoski & Davidson, 2011; McNay & Recknagel, 2011; M. C. Morris, 2012), which are all typical of the 'Western diet', have been associated with poorer late-life cognitive outcome including dementia. Similar findings have been reported by individual studies on carbohydrate intake also linked to this type of diet (Miller, Bannerman, Daniels, & Crotty, 2006; Roberts et al., 2012). In contrast, high protein and high fat intake may be protective both in terms of level of cognitive function short of dementia and risk of dementia (Miller, et al., 2006; Roberts, et al., 2012). The Mediterranean diet combines beneficial patterns of micro- and macronutrient intake and a review of the literature concludes that it is consistently linked to reduced rates of cognitive decline short of dementia and reduced dementia risk (Polidori, et al., 2010).

Intake of saturated fats is typically high in type 2 diabetes and may even predict risk of future diabetes (Lichtenstein & Schwab, 2000). In a prospective cohort study of females with type 2 diabetes, high baseline saturated and trans-fat intake predicted steeper two-year cognitive decline short of dementia (Devore et al., 2009). A rare RCT in older patients with type 2 diabetes also found that lifestyle intervention with dietary restriction and exercise improved cognitive function over similar length of follow-up. For instance, MMSE scores improved by 1.9 points (Yamamoto, Yamanaka, Takasugi, et al., 2009). Importantly, because type 1 diabetes patients are also cognitively affected (Brands et al., 2006; Ryan, Williams, Finegold, & Orchard, 1993) despite not typically following detrimental dietary patterns, poor nutrition (as well as obesity) are presumably not the main contributors to the accelerated cognitive decline observed in type 2 diabetes.

2.6.5 Socioeconomic status

Peoples' relative position in society and their access to resources define their socioeconomic status (SES), commonly estimated by educational achievement, parents' SES, household income, occupation, number of assets or post code. SES is a largely non-modifiable risk factor which tends to remain relatively stable during the life course. Cross-sectional and prospective studies have linked lower SES to poorer late-life cognitive function short of dementia (Jefferson et al., 2011; Koster et al., 2005; Marengoni, Fratiglioni, Bandinelli, & Ferrucci, 2011), as well as to an increased risk of cognitive decline short of dementia and of dementia diagnosis (Marengoni, et al., 2011; Zeki Al Hazzouri, et al., 2011). The mechanisms responsible may involve deprived backgrounds predisposing individuals to maladaptive behaviour and harmful lifestyles. However, associations of SES with late-life ability are also confounded by strong associations of SES with peak pre-morbid ability, which become apparent in the aforementioned list of estimators. Patients with type 2 diabetes typically have lower SES than non-diabetic older adults (Tamayo, Herder, & Rathmann, 2010) and it has been suggested that low SES stands at the outset of the "chain of risks" (Anstey, 2008, p. 284) contributing to the cognitive decline seen in type 2 diabetes.

2.6.6 Alcohol

Alcohol has both hypoglycaemic and hyperglycaemic effects. In the short-term, reactive hypoglycaemia may be caused by a rise in insulin and a decrease in release of glucose from glycogen stores, up to a depletion of these stores, and negatively affects cognitive function (van de Wiel, 2004). Moderate consumption has been associated with higher level of cognitive function, including in people with type 2 diabetes (Fan, O'Donnell, Singh, Pungan, & Perlmutter, 2008; Townsend, Devore, Kang, & Grodstein, 2009). Cross-sectional analyses may be restricted by ‘sick quitters’ who quit drinking due to poor health (Neafsey & Collins, 2011), but are supported by prospective investigations. A systematic review of 23 studies concluded that moderate consumption reduces the risk of any type of dementia by 37% and of Alzheimer’s Disease by 43% in the general population (R. Peters, Peters, Warner, Beckett, & Bulpitt, 2008), although associations with risk of vascular dementia appear to be more inconsistent (Anstey, Mack, & Cherbuin, 2009; R. Peters, Peters, et al., 2008). For instance, in two studies of the general population, moderate consumption or consumption of ≥1 drink/day was associated with 15% to 60% reduced risk of ‘steep’ late-life cognitive decline short of dementia over follow-ups of two to four years (Espeland et al., 2005; Stampfer, 2006). Yet, findings in another seven-year prospective investigation were mixed, with significant associations restricted to a single of six cognitive domains (Zanjani, Downer, Kruger, Willis, & Schaie, 2013), and one recent relatively large meta-analysis of 143 studies found that moderate drinking was unrelated to cognitive decline (Neafsey & Collins, 2011). A rare investigation in type 2 diabetes also did not establish significant associations of moderate drinking with subsequent rates of cognitive decline during four years of follow-up (Townsend, et al., 2009). Given that alcohol consumption appears to remain moderately stable over time (Hansell et al., 2008), this suggests that a moderate alcohol consumption has long-term protective effects on cognitive function, for instance due to a reduction in the risk of plaque deposits in arteries, improvements in insulin-sensitivity and protection from blood clotting (Zakhari, 1997). Moderate levels of ethanol have also been linked *in vitro* to reduced beta amyloid concentrations (M. A. Collins et al., 2010), and the non-alcoholic components of drinks may have antioxidant, anti-inflammatory and vasorelaxant effects (Gupta & Warner, 2008).

Long-term excessive alcohol consumption appears to increase the risk of dementia (Deng et al., 2006; Saunders et al., 1991), although the evidence for effects on cognitive decline short of dementia has been mixed (Anstey, et al., 2009; Y. Lee et al., 2010). Excessive consumption increases the risk of type 2 diabetes (Pietraszek, Gregersen, & Hermansen, 2010) and a rare cross-sectional investigation of this topic reported that history of alcohol abuse in type 2 diabetes enhanced the detrimental effects of each condition on the level of mid-life cognitive function (Hudetz & Warltier, 2007).

2.7 Chapter summary

A number of factors have been identified in the literature as potentially contributing to increased risk of poorer cognitive outcome in later life in the general population and in people with diabetes. The differential prevalence of some of these risk factors in people with type 2 diabetes and the general population may in part account for the increased risk of cognitive decline in diabetes described in Chapter 1. Potentially modifiable risk factors for cognitive decline include factors associated with metabolic function, factors which contribute to vascular disease, and individual differences in behaviour. Other factors, such as genetic predisposition or socioeconomic status, are non-modifiable. Many are inter-related in various dynamic pathways, which may also evolve over the course of the lifetime. Consequently, research aimed at identifying potential causal determinants of cognitive ageing is extremely complex and difficult, and this applies both to the general population and to people with type 2 diabetes. This thesis will focus on two selected risk factors in their associations with cognitive decline. The evidence from the previous literature on these risk factors is presented in the next chapter.

Chapter 3: Association of macrovascular disease and hypoglycaemia with cognitive function: literature review and thesis aims

In this chapter, evidence on the association of two of the main risk factors considered in this thesis (macrovascular disease and severe hypoglycaemia) with cognition is reviewed in detail, using a systematic approach to searching the literature for relevant epidemiological studies. Aims and objectives of the thesis are presented in the final part of the chapter.

3.1 Hypoglycaemia

3.1.1 Symptoms and consequences

When insulin levels do not match food intake or activity, when insulin sensitivity is increased or insulin clearance is decreased, blood glucose drops to insufficient levels in people with diabetes. The risk of hypoglycaemia poses a considerable threat to people with diabetes. Biochemical hypoglycaemia occurs at around <3.8mmol/l, but symptoms do not arise until levels of around 3.3mmol/l (Graveling & Frier, 2010). These include tremulousness, palpitations, anxiety, excessive sweating, hunger, paresthesias, confusion, seizures or loss of consciousness resulting in diabetic coma (Cryer, et al., 2003). In patients with type 2 diabetes, ataxia and visual disturbance are particularly common (Graveling & Frier, 2010). Negative impacts of acute hypoglycaemia on cognitive function, as determined in studies experimentally inducing episodes of hypoglycaemia, appear well-established (Amiel et al., 1991; Bremer et al., 2006; Cox, Gonder-Frederick, Kovatchev, Julian, & Clarke, 2000; Hvidberg et al., 1996; Wirsén, Tallroth, & Lindgren, 1992). Cognitive deficits usually arise at peripheral glucose levels of around 2.6 to 3.0 mmol/l (Warren & Frier, 2005). Compared with younger patients with diabetes, symptoms are generated at lower blood glucose levels in older patients with type 2 diabetes, and cognitive symptoms arise at earlier time points. Consequently, cognitive deficits (limiting a patient's ability to respond) and hypoglycaemia (demanding urgent action by the patient) may occur concurrently (Graveling & Frier, 2010).

Severe hypoglycaemia (SH) is typically defined as a symptomatic episode which requires the help from another person to effect recovery. Two to 4% of such episodes result directly in the death of the patient (Diedrich, Sandoval, & Davis, 2002). Hypoglycaemia of any severity has further been estimated to account for five fatal road accidents per year in the UK alone (Amiel, Dixon, Mann, & Jameson, 2008). Consequently, recurrent episodes may heighten fear of hypoglycaemia and reduce quality of life (Barendse, Singh, Frier, & Speight, 2012). Particularly nocturnal hypoglycaemia may be a cause of worry (Munro, Barnett, Brod, & Peyrot, 2013).

3.1.2 Difficulty of diagnosis, and prevalence

Recurrent hypoglycaemia gradually induces a change in the glycaemic threshold for the generation of symptoms. This has been termed 'impaired awareness of hypoglycaemia' (IAH) (Cryer, 2001). Due to IAH, biochemical hypoglycaemia may then occur with reduced intensity of symptoms or changes in symptom profiles. Around 25% of patients with type 1 diabetes and around 8% to 10% of insulin-treated patients with type 2 diabetes appear to be affected by either partial or absent awareness of hypoglycaemia (Graveling & Frier, 2010), which has been associated with a substantial increase in the risk of future hypoglycaemia (Bakatselos, 2011).

In addition to IAH, recurrent episodes of hypoglycaemia cause a progressive deficiency in the ability of the autonomic central nervous system (CNS) to counter-regulate hypoglycaemia. This has been termed 'hypoglycaemia-associated autonomic failure (HAAF) (Cryer, 2001). In both humans and in animal models, even a single episode of hypoglycaemia without loss of consciousness has been found to reduce subsequent counter-regulation (Languren, Montiel, Julio-Amilpas, & Massieu, 2013; Tkacs, Pan, Raghupathi, Dunn-Meynell, & Levin, 2005). A vicious cycle of impaired symptom generation and increased risk of hypoglycaemia follows. Although both IAH and HAAF are initiated by recurrent hypoglycaemia, both are not always linked. Avoidance of hypoglycaemia may improve IAH while the counter-regulatory response by the central nervous system may remain poor (Graveling & Frier, 2010).

In addition to these issues, which complicate the response by patients to ensure reversion to normoglycaemia as well as the accurate identification of hypoglycaemia in studies using self-report data, many older patients may also lack knowledge of the specific symptoms of hypoglycaemia (Thomson, Masson, Leeming, & Boulton, 1991). When symptoms are atypical, episodes may further be wrongly attributed to other age-related disorders, such as transient ischaemic attacks (TIA) (Jaap, Jones, McCrimmon, Deary, & Frier, 1998). Self-report is further limited by false positives when symptoms are wrongly attributed to hypoglycaemia by patients or physicians. Alternatives to self-report, such as continuous glucose measurement (UK Hypoglycaemia Study Group, 2007) or analysis of hospital records (Whitmer, Karter, Yaffe, Quesenberry, & Selby, 2009) are rarely used and have their own limitations. The use of hospital records, for instance, may not be advisable in research of associations with cognitive function, because hypoglycaemia is relatively common in hospital settings and particularly in seriously ill individuals (Graveling & Frier, 2009, 2010). Data based on hospital records may further be subject to potential influences by physician-dependent tendency to record medical problems. A physician who is sensitive to a patient’s cognitive deficits may be more likely to also record the experience of hypoglycaemia compared with another physician who is less concerned with a patient’s state of cognitive function. Continuous glucose monitoring may offer an alternative method of ascertaining hypoglycaemia, but is difficult to apply to long-term investigations of large cohorts.

Hypoglycaemia occurs less frequently in type 2 than in type 1 diabetes. However, with SH in people with type 2 diabetes most commonly occurring in those on insulin treatment (annual prevalence 3% to 15% depending on the duration of treatment (UK Hypoglycaemia Study Group, 2007)), prevalence is overall equivalent amongst people with type 1 and those with type 2 diabetes when groups are matched for duration of insulin treatment (Cryer, 2001). In one UK study, 84% of insulin-treated patients with type 2 diabetes self-reported at least one episode of any severity since diagnosis (Munro, et al., 2013). However, oral treatment with sulfonylureas also confers a considerable risk of SH (UK Hypoglycaemia Study Group, 2007). Hypoglycaemia occasionally occurs even in diet-controlled individuals in postprandial or post-operative phases, with an annual prevalence of SH in this patient group of around 0.1% (UKPDS, 1998).

3.1.3 Counter-regulation and brain response to hypoglycaemia

The body responds to hypoglycaemia with an increase in heart rate and systolic blood pressure as well as secretion of glucagon, pancreatic polypeptide, cortisol, norepinephrine, epinephrine and growth hormone (Cryer, 2001; Cryer, et al., 2003). Glucagon, for instance, facilitates the production of glucose in the liver, which increases blood glucose levels. Epinephrine decreases insulin secretion, stimulates lipolysis and glucose synthesis (Languren, et al., 2013). Glucagon and epinephrine act in the short-term and at relatively mild hypoglycaemia (around 3.9mmol/l), whereas counter-regulation by cortisol and growth hormone occur at lower blood glucose levels and are delayed by around six hours (Cryer, 2001; Languren, et al., 2013).

Brain glucose levels are generally around 30% of those in the periphery (Convit, 2005). Hypoglycaemia leads to an increase in glucose transport across the blood-brain barrier, for instance through up-regulation of GLUT1 and GLUT3. Additionally, the brain uses glycogen stored in astrocytes, overall 'super-compensating' for glucose deficits (McNay & Cotero, 2010). Consistent with the 'selfish brain theory' (A. Peters et al., 2004), the remaining parts of the body limit their energy use by reducing insulin secretion and increasing lipolysis and proteolysis, which enable the degradation of fats and proteins (Cryer, 2001; Cryer, et al., 2003; Diedrich, et al., 2002). Consequently, and presumably because cortisol and norepinephrine act as cognitive enhancers (McNay & Cotero, 2010), hypoglycaemia has occasionally been linked to improved cognitive ability at least in rodents (McNay & Sherwin, 2004).

In the long-term, recurrent episodes of hypoglycaemia are rarely linked to overt neural damage, changes in cerebral activity and sensory deficits in humans and rodents (Akyol, Kiylioglu, Bolukbasi, Guney, & Yurekli, 2003; Puente et al., 2010). When significant associations *are* reported, these typically become apparent only following profound episodes of hypoglycaemia (blood glucose <1.5mmol/l) which often involve coma (Ryan, 2009).

Despite this, long-term cognitive deficits, which may be caused by *subclinical* brain damage, are important to consider. In this chapter, evidence on the association of hypoglycaemia with cognition in people with diabetes is considered. This is done separately for type 1 and type 2 diabetes, because of differences between the two conditions; type 1 diabetes patients are typically less affected by co-morbidities (Plotnikoff et al., 2007) and are usually younger than people with type 2 diabetes, and so (in contrast to the latter) are not in a 'crucial period' at which the brain may have increased vulnerability to insults because it undergoes change (Biessels, et al., 2008). Evidence from studies on type 1 diabetes is considered first, followed by a systematic review of studies on type 2 diabetes (the focus of subsequent analyses in this thesis).

3.1.4 Hypoglycaemia and cognitive function in people with type 1 diabetes

Cross-sectional associations between a history of SH and lower level of cognitive function are well-established in children with type 1 diabetes (Blasetti et al., 2011). In adults, one relatively small investigation revealed no cross-sectional associations between a lifetime history of SH or frequency of SH and performance on a variety of cognitive tests in young people with type 1 diabetes. Even a history of coma due to SH was unrelated to cognitive function (Ferguson, et al., 2003).

In terms of cognitive change, a small cross-sectional analysis of patients with type 1 diabetes found that those who had a history of at least five episodes of SH experienced a steeper decline between estimated peak pre-morbid ability and current level of cognitive function (Langan, Deary, Hepburn, & Frier, 1991). In the same sample, frequency of SH was also significantly associated with the rate of estimated lifetime cognitive decline (Deary, Langan, Graham, & Hepburn, 1992). A trend just short of statistical significance was reported for associations of SH frequency and estimated lifetime cognitive decline in a study of younger to middle-aged patients with any type of diabetes (Lincoln, Faleiro, Kelly, Kirk, & Jeffcoate, 1996). Another study further found that patients who had recently suffered an episode of SH had consistently lower processing speed and executive functioning when assessed 1.5, 9 and 30 days following the episode compared with patients who had been free of SH in the previous year. Little evidence was found for cognitive improvement in the SH group over the course of the 30 day period (Strachan, Deary, Ewing, & Frier, 2000). In the Diabetes Control and Complications Trial (DCCT) of adults with type 1 diabetes which investigated consequences of intensified glycaemic control, episodes of SH were unrelated to nine-year cognitive change (Austin & Deary, 1999). A re-analysis of the data together with the follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) similarly found no associations between SH and cognitive change over an 18-year period between young adulthood and middle-age (The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group, 2007). The conclusions drawn on the basis of DCCT/EDIC may be limited by its selection for individuals with low risk of hypoglycaemia and the wide age range of participants (13 to 39 years at baseline), but are supported by a smaller Swedish trial, the Adults in Stockholm Diabetes Intervention Study (SDIS). Here, participants in two treatment arms differed in their frequency of SH, but had similar levels of cognitive function at the end of a ten-year follow-up (Reichard, Pihl, Rosenqvist, & Sule, 1996).

Overall, the evidence from adults with type 1 diabetes suggests that the experience of severe hypoglycaemia which is short of coma may be unrelated to cognitive function or cognitive change. This does not mean that older adults with type 2 diabetes, who experience a ‘crucial period’ of brain development, are necessarily similarly unaffected.

3.1.5 Hypoglycaemia and cognitive function in people with type 2 diabetes

A systematic search of the literature was performed in order to identify all previous cross-sectional and prospective analyses of hypoglycaemia and cognition in older patients with type 2 diabetes investigating hypoglycaemia either as a risk factor for long-term poorer cognitive outcome or as a potential consequence of lower ability.

Search strategy

Titles, abstracts and keywords (.mp) of studies indexed in Medline between 1946 and the third week of April 2013 were searched. The terms "type 2 diabetes", "non-insulin-dependent" and "NIDDM" were combined with a Boolean OR operator, as were "hypoglyc*mia", "low blood sugar", "low blood glucose". As the outcomes of interest, the keywords "cognit*", "intelligence" and "abilit*" were again searched with an OR operator. The three searches were combined using the AND operator. Additionally, reference lists of key papers and reviews were examined, and experts in the field were consulted.

Selection of studies

Studies were selected for inclusion if they fulfilled the following criteria: a) original studies b) studies of human type 2 diabetes patients or older adults (mean age >55 years) with unspecified diabetes, c) ascertainment of hypoglycaemic episodes of any severity and using any type of method, d) application of neuropsychological tests, brain imaging, clinical evaluation of dementia or self-report to ascertain cognitive function, and e) reporting of data linking hypoglycaemia and cognitive function. Case studies and reviews of the literature were excluded from the review.

Data extraction

The titles and abstracts of all studies identified by the search were screened. Full articles of those which appeared to meet the criteria or where this could not be ascertained on the basis of the abstract alone were obtained and reviewed for inclusion. Data from studies which were included in the review were extracted, with focus on the following topics: study design, total number of participants, age at baseline (range or mean), measurement of cognitive function, ascertainment of hypoglycaemia, treatment of hypoglycaemia as an dependent variable or an independent variable, main findings including effect sizes and p-values or confidence intervals where available, and study limitations. Extracted data from all included studies were tabulated to provide an overview of the evidence.

Results

Following removal of duplicates the search resulted in 120 papers (Figure 3.1). All abstracts were evaluated, and the full texts of 19 studies were accessed. Of these, 12 studies were found to meet the inclusion criteria. One additional study was published after the search had been performed (Yaffe et al., 2013) and is also included in this review. The designs and main findings of all included studies (total n=13) are summarised in Table 3.1 and Table 3.2.

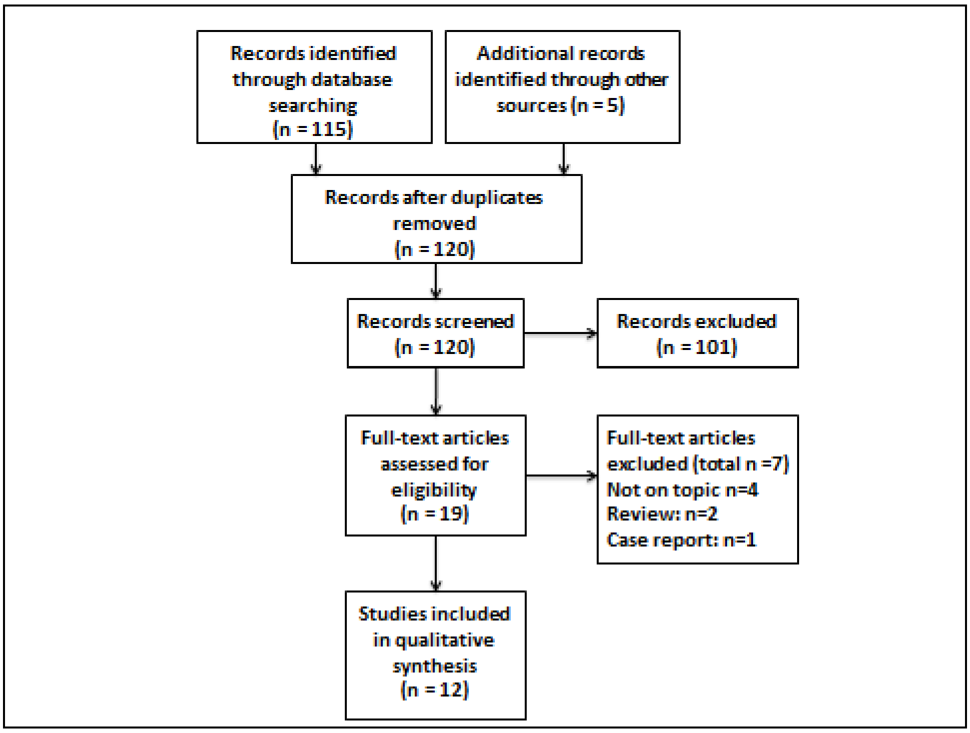

Figure 3.1: Systematic literature search on hypoglycaemia and cognition in type 2 diabetes

Table 3.1: Summary of studies identified in systematic search on hypoglycaemia and cognition in older adults with diabetes

	Sample	Design	Hypo as IV*	Hypo as DV**	Total N	Age at baseline	Measurement of hypoglycaemia	Measurement of cognitive function
Bruce et al. (2001)	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Cross-sectional	Yes		63	>70 years (mean 75 years)	1. Self-reported history of hypoglycaemia 2. Hospital records for admissions for hypoglycaemia	'Probable dementia; determined from combination of MMSE and IQCODE
Hissa et al. (2002)	Type 2 diabetes patients (all MMSE>26), Brazil	Cross-sectional	Yes		44	35-78 years (mean 58 years)	1. Self-reported history of hypoglycaemia and self-reported requirement of assistance 2. Questionnaire on severity of hypoglycaemic episode on scale (score 0 – 15)	P300 component on ERP
McGill et al. (2006)	GEMINI (hypertensive patients with type 2 diabetes), USA	5-month prospective RCT	Yes		1235	36-85 years (mean 61 years)	Self-reported 'hypo' within last month	Self-reported cognitive problems within last month
Bruce et al. (2009)	Fremantle Diabetes Study (patients with any type of diabetes), Australia	1. Cross-sectional 2. 2-year prospective 3. 5-year prospective	Yes	Yes	302	>70 years (mean 76 years)	1. Self-reported medical assistance and/or unconsciousness. 2. Episodes rated by medical staff as 'proven', 'probable', 'uncertain'. 'Doctor-	1. MMSE, CDR, IQCODE 2. neuropsychological assessment. MCI defined as CDR=0.5 without dementia; 'cognitive

							verified' defined as probable/proven episode. 3. Codes for ambulance or emergency treatment for hypoglycaemia in hospital records (HSH)	decline' defined as conversion between normal cognition, MCI and dementia
De Galan et al. (2009)	ADVANCE (type 2 diabetes patients in 20 countries), Australia	Median 5-year prospective RCT	Yes	Yes	11 140	>55 years (mean 66 years)	1. Mild hypoglycaemia defined as self-treated 2. SH defined as blood glucose <2.8mmol/l or requiring external assistance	1. MMSE 'Normal': >28 'Mild dysfunction': 24-27 'Severe dysfunction': <24 2. Neurospsych. assessment of 'severe' group
Whitmer et al. (2009)	Type 2 diabetes patients, USA	1. 5-year prospective (1980-1985) assessment of hypo; dementia during 4 years (2003-2007); 2. 18-year prospective (1980 – 2002) assessment of hypo; dementia during 4 years (2003 – 2007)		Yes	16 667	>55 years (mean 65 years)	Codes for emergency department treatment or hospitalisation for any hypoglycaemia	Codes for any dementia in inpatient and outpatient medical records
Aung et al. (2011)	Edinburgh Type 2 Diabetes Study (type 2 diabetes	Cross-sectional	Yes		1066	60-75 years (mean 68 years)	Self-reported history of episode requiring assistance	MMSE; 7 cognitive tests

	patients), Scotland							
Feil et al. (2011)	Veterans with diabetes (patients with any type of diabetes), USA	Cross-sectional (2-year period)	Yes		497 900	≥65 years (44% ≥75 years)	Codes for outpatient treatment, emergency department and inpatient records for hypoglycaemia on hospital records	1. Codes for dementia on hospital records 2. Codes for 'cognitive impairment' (MCI; amnestic memory deficits; other cognitive disorder) on hospital records
Hewitt et al. (2011)	Diabetes patients (any type) in RCT (data used from 1 treatment arm only), UK	Cross-sectional	Yes		1047	>75 years (mean 81 years)	Self-reported history of 'low blood sugar' or 'hypo'	MMSE<24 versus MMSE ≥24
Launer et al. (2011)	ACCORD-MIND (type 2 diabetes patients), North America	40-month prospective RCT (3.3 years)	Yes		2977	≥ 55 years (mean 63 years)	Cites reference reporting that the intensive treatment arm had increased risk of SH	1. MMSE, DSC, RAVLT 2. Imaging (MRI)
Lin & Sheu (2013)	Diabetes patients (any type); all previously free of hypoglycaemia, Taiwan	1. 3-year prospective hypo ascertainment 2.Subsequent 4-year prospective dementia ascertainment		Yes	15 404	>45 years (mean 64 years)	Codes for any hypoglycaemia from inpatient and outpatient medical records	Codes for any dementia from inpatient and outpatient medical records
Punthakee et al. (2012)	ACCORD-MIND (type 2 diabetes patients),	Mean 3.5-year prospective		Yes	2957	≥ 55 (mean 62 years)	1. SH defined as self-reported <2.8 mmol.l or symptoms that resolved with use of glucose or	MMSE, RAVLT, Stroop, DSC (main outcome)

	North America						similar 2. HMA defined as episode requiring medical assistance (hospitalisation; care in emergency department/ by emergency personnel) 3. HAA defined as episode requiring any assistance (from medical or non-medical persons)	
Yaffe et al. (2013)	Health, Aging and Body Composition Study (Health ABC) (patients with any type of diabetes; dementia-free at baseline), USA	12-year prospective	Yes	Yes	783	70-79 years (mean 74 years)	Codes for hospital admission for hypoglycaemia on hospital records	1. Codes for any type of dementia on hospital records 2. MMSE administered every 2 years

Hypo, hypoglycaemia; RCT, randomised controlled trial; MMSE, Mini Mental State Examination; IQCODE, Informant Questionnaire on for Cognitive Decline in the Elderly; CDR, Clinical Dementia Rating Scale; neuropsych., neuropsychological; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; DSC, Digit Symbol Coding; RAVLT, Rey's Auditory Verbal Learning Test; IV, independent variable; DV, dependent variable; ERP, event-related potential. *study investigated potential of hypoglycaemia as a risk factor for poorer cognitive function; **study investigated potential of poor cognitive function as a risk factor for hypoglycaemia.

Table 3.2: Main findings from studies identified in systematic search

	Main findings	Limitations
Bruce et al. (2001)	No difference in prevalence of 'probable dementia' in people with hypoglycaemia and others (in separate analyses for hospital admission for hypoglycaemia and self-reported hypoglycaemia) (p=0.63)	'Probable dementia' determined from screening instruments
Hissa et al. (2002)	1. No difference in P300 between patients with history of hypoglycaemia and others (p=0.31) 2. No relationship between P300 component and frequency of hypoglycaemic episodes (p=0.28)	Small n; wide age range
McGill et al. (2006)	One treatment arm had beneficial effects on risk of hypoglycaemia over 5 months compared to the other group (p=0.02), but change in cognitive deficits was equivalent in both treatment groups (p=0.43; neither experienced change in cognitive deficits)	Use of self-report for both predictor and outcome; wide age range
Bruce et al. (2009)	1. Cross-sectional unadjusted analyses: Increased prevalence of self-reported SH in patients with MCI (14%) and dementia patients (16%) compared with remaining sample (4%) (both p<0.05)Similar results for doctor-verified measures of hypoglycaemia (all p<0.05). Link between HSH and dementia (p<0.001) but not MCI 2. Cross-sectional analyses adjusted for clinical and demographic covariates: for group with MCI, OR 2.96, p=0.040 for self-reported SH, OR 5.10, p=0.011 for doctor-verified hypoglycaemia and OR 9.65, p=0.012 for HSH). Findings non-significant for dementia (p>0.10) 3. Prospective analyses: No association between any baseline hypoglycaemia measure and risk of 'cognitive decline' (p=0.09 for all doctor-verified hypoglycaemia, in direction of higher prevalence of hypoglycaemia in 'no decline' (44%) than in 'decline' group (26%)); baseline dementia predicted 202% increased risk of HSH during follow-up (p=0.037)	Only n=33 'declined' during follow-up; short follow-up; loss of power due to categorisation of cognitive outcome; potentially reduced validity of self-report in patients with dementia
De Galan et al. (2009)	1. 'Mild' and 'severe' dysfunction (versus 'normal') at baseline predicted 39% and 286% increased risk of SH (but not 'mild' hypoglycaemia) in unadjusted analyses (both p<0.0001). Significance was lost when age, sex, disease duration, education were controlled for in 'mild dysfunction' group (p=0.32) and attenuated to 186% increased risk in 'severe dysfunction' group (p=0.003) 2. Each 1 point lower baseline MMSE predicted 10% increased risk of SH (p=0.001) 3. Intervention led to increased frequency of SH in one treatment arm, but no difference between treatment arms in cognitive decline or risk of dementia (data not shown) 4. Intervention increased risk of SH to similar extents in all 3 cognitive groups	Only used MMSE with neuropsychological follow-up; low incidence of SH (baseline exclusion of patients requiring insulin treatment); hypoglycaemia only treated as an outcome; loss of power due to categorisation; 'data not shown' for some findings; did not report outcome of neuropsychological assessment of 'severe dysfunction' group so prevalence of dementia

		unclear
Whitmer et al. (2009)	1. In both 5-year and 18-year analyses: hypoglycaemia (≥1 versus none) increased risk of subsequent dementia (e.g., for 5-year analysis: 2.4% increased risk per year of follow-up, 95% CI 1.7%, 3.0%). Even greater risk for ≥2 episodes and ≥3 episodes 2. Similar results when only including ICD codes from emergency departments	Use of hospital records (hypoglycaemia may be common in hospital settings)
Hewitt et al. (2010)	No difference in prevalence of 'hypo' between 'cognitively impaired' and 'unimpaired' individuals (p=0.08)	Unclear definition of hypoglycaemia; assessed cognition only using MMSE
Aung et al. (2011)	1. Patients with ≥1 episode of SH had lower cognitive function compared with SH-free individuals 2. These patients also experienced steeper estimated lifetime decline (determined by adjustment for estimate of peak pre-morbid ability) 3. Linear relationship between number of episodes of SH and level of cognitive function	
Feil et al. (2011)	Association between ≥1 episodes and increased prevalence of dementia and cognitive impairment (unadjusted OR 2.42 and 1.71 respectively compared with individuals free of both dementia and cognitive impairment). Adjustment for range of demographic and clinical covariates led to attenuation of OR to 1.84 and 1.22 respectively) (all p<0.001)	Use of hospital records; 'cognitive impairment' could include a range of disorders
Launer et al. (2011)	1. No difference in 40-month cognitive decline between intensive treatment and standard treatment groups (p=0.23 to p=0.93 for individual cognitive tests), despite increased frequency of SH in intensive treatment group 2. Trend for steeper 20-month decline on DSC in intensive treatment group (p=0.076)	Failed to directly associate SH with cognitive decline
Lin & Sheu (2012)	1. Association between ≥1 episodes over 3 years and increased risk of subsequent dementia by 276% (p<0.001) in unadjusted analyses and by 60% (p=0.002) when adjusted for age, sex 2. Number of episodes predicted risk of dementia (p<0.001; adjusted for age, sex)	Use of hospital records; wide age range so may have included type 1 diabetes
Punthakee et al. (2012)	1. HMA group had lower baseline cognitive test performance compared with group free of HMA (p<0.0001 to p=0.002 for individual cognitive tests) 2. Lower baseline cognitive function predicts increased risk of first-ever HMA and HAA (largest effect observed for DSC: in largely unadjusted analyses, 5-point lower DSC predicted 13% increased risk of HMA (95% CI 8% to 18%)	Unclear reason for focus on DSC as main outcome, and for choice of the specific cut-point

	and 11% increased risk of HAA (95% CI 7% to 15%) 3. Association between 20-month decline in DSC and risk of any first-ever hypoglycaemic episode in subsequent 22 months (for low baseline ability individuals only)	
Yaffe et al. (2013)	1. Association between ≥1 episode and risk of dementia (adjusted for age, sex, education, ethnicity, diabetes prevalent at baseline, treatment, HbA1c, APOE e4 status, and baseline MMSE: OR 2.09, 95% CI 1.00, 4.35). Finding survived additional adjustment for change in MMSE scores prior to hypoglycaemic episode 2. Patients with dementia at increased risk of subsequent hypoglycaemia (adjusted for age, sex, education, ethnicity, diabetes prevalent at baseline, treatment and baseline MMSE: OR 3.10, 95% CI 1.46, 6.58)	Use of hospital records

DSC, Digit Symbol Coding; OR, odds ratio; APOE e4, apolipoprotein e4 allele; CI, confidence interval; SH, severe hypoglycaemia; MMSE, Mini Mental State Examination.

Overview of included studies

The 13 included studies reported data from 11 cohorts and a total of 536 585 participants, a majority of whom (n=497 900; 89%) came from a single analysis of US Veterans (Feil et al., 2011). Seven studies included only patients with diagnosis of type 2 diabetes; the remaining 6 included individuals with any type of diabetes, but all had mean ages >55 years so that a majority of subjects will have suffered from type 2 diabetes. All studies included male and female participants. A total of 5 studies had only cross-sectional designs, whereas the remaining 8 studies were prospective investigations. Three of the prospective studies were randomised controlled trials on the effects of antihypertensive or anti-diabetic treatment; all of the remaining investigations were observational cohort studies. Seven studies treated hypoglycaemia as an independent variable, i.e. a potential predictor of cognitive outcome, 3 studies investigated hypoglycaemia as a dependent variable, and 3 carried out both types of analyses. The studies were heterogeneous with respect to the assessment of hypoglycaemia. Measurements ranged from self-reported potentially very mild ‘hypos’ to hospital records of episodes requiring emergency treatment. The latter category may have included episodes resulting in coma, although none of the studies specifically set out to investigate such episodes. A total of 5 studies reported data on clinically diagnosed dementia (most of which were based on hospital records, n=4) either with or without additional cognitive data, 1 study included a screening instrument which was followed up by neuropsychological assessment to identify people with ‘severe cognitive dysfunction’ (although prevalence of dementia in this group was unclear), 5 studies relied on cognitive tests or screening instruments without neuropsychological assessment or consultation of medical records, 1 measured brain activity and 1 used a self-report measure of cognitive problems. Due to the described heterogeneity between the studies in terms of study design and cognitive outcome, a meta-analysis of data to derive overall effect sizes on associations between hypoglycaemia and cognitive function was not possible. Instead, the findings of the included studies are described with the aim to offer an overall picture of the evidence on links of hypoglycaemia with cognition.

Findings from included studies

Cross-sectional evidence on association between hypoglycaemia and cognitive ability

The baseline analysis of the Edinburgh Type 2 Diabetes Study revealed statistically significant associations between a self-reported lifetime history of SH and lower global cognitive ability as well as lower scores on several cognitive domains (Aung et al., 2012). A further UK study of around 1000 individuals reported statistically non-significant trends (p=0.08) for increased frequency of self-reported history of ‘low blood sugar/ ‘hypo’’ in older adults with type 2 diabetes and reduced cognitive function (MMSE<24) compared with higher-functioning diabetes patients. The results may have been weakened by the measure of hypoglycaemia, which may have included mild episodes (Hewitt, Smeeth, Chaturvedi, Bulpitt, & Fletcher, 2011). In the Action to Control Cardiovascular Risk in Diabetes- Memory in Diabetes (ACCORD-MIND) trial, patients with a history of a hypoglycaemic episode which required medical assistance had a lower level of cognitive function compared with the remaining sample (Punthakee et al., 2012). Similar findings were reported in a cross-sectional study of almost 500 000 US veterans. Individuals with hospital records showing reduced cognitive function short of dementia were 71% more likely to also have a record for hypoglycaemia compared with individuals without codes for either dementia or cognitive deficits (Feil, et al., 2011).

The Fremantle Diabetes Study also found increased prevalence of a history of SH (self-reported or verified by a clinician) in patients with reduced level of late-life cognitive function short of dementia. For instance, the likelihood of a self-reported history of SH in this group was three-fold even after multivariable adjustment compared with cognitively unimpaired participants (Bruce et al., 2009). In contrast, an analysis of a relatively smaller baseline sample of the same cohort had failed to establish cross-sectional associations between a self-reported history of any hypoglycaemia or hospital admission for hypoglycaemia and the presence of reduced level of cognitive function (Bruce, Harrington, Davis, & Davis, 2001). In addition, one study found that the P300 component thought to reflect cognitive processing was unrelated to a self-reported history of or frequency of hypoglycaemia (Hissa, D'Almeida, Cremasco, & de Bruin, 2002).

Compared with these slightly mixed findings, the evidence appears more consistent for associations with frank dementia. In the Fremantle Diabetes Study, a lifetime history of SH was more prevalent in patients with diagnosed dementia compared with dementia-free individuals at least in unadjusted analyses. For instance, 21% of the dementia patients had a doctor-verified severe episode compared with 3% of the cognitively ‘normal’ participants (Bruce, et al., 2009). In the study of US veterans, the prevalence of dementia in individuals who experienced a hypoglycaemic episode over a two-year period was also almost two-fold compared with hypoglycaemia-free individuals when demographics as well as anti-diabetic treatment, microvascular and macrovascular disease were controlled for (Feil, et al., 2011)

Evidence on reduced cognitive ability as a risk factor for hypoglycaemia

Cross-sectional evidence does not reveal the temporal relationship between risk factor and outcome, but studies with prospective designs can help to resolve this issue. The evidence for SH as a consequence of reduced cognitive ability in type 2 diabetes appears relatively well-established. In the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation

(ADVANCE) trial, 'severe cognitive dysfunction' at baseline (which may or may not have included patients with dementia) predicted 186% increased risk of SH over five years even when age, sex, disease duration and treatment mode were accounted for. Additionally, each one unit lower MMSE at baseline was associated with a 10% increased risk of SH during follow-up (de Galan et al., 2009). In ACCORD-MIND, a lower performance on a more detailed neuropsychological test battery also predicted increased risk of first-ever hospital admission for hypoglycaemia. Additionally, for a group with relatively low initial processing speed, a steeper rate of 20-month cognitive decline predicted increased risk of SH during the subsequent 22 months (Punthakee, et al., 2012). Clinical diagnosis with dementia at baseline also increased patients' risk of hospital admission or emergency treatment for hypoglycaemia by around two-fold in the Fremantle Diabetes Study (Bruce, et al., 2009) and by three-fold in the US Health, Aging and Body Composition Study (Health ABC) (Yaffe, et al., 2013).

Evidence on hypoglycaemia as a risk factor for subsequent cognitive decline/dementia

In terms of SH as a risk factor for cognitive decline, the balance of evidence is less clear. In the range of cognitive impairment short of dementia, the baseline analysis of the Edinburgh Type 2 Diabetes Study showed associations between a baseline history of SH and steeper estimated lifetime decline on a global ability factor and in several cognitive domains. This was determined in cross-sectional analyses of current late-life cognitive test scores with adjustment for an estimate of peak pre-morbid ability (Aung, et al., 2012). These findings indicate at least an acceleration of cognitive decline pre- to post-hypoglycaemic episode, but are at odds with findings from the only three studies on prospectively ascertained late-life cognitive decline which is short of dementia (de Galan, et al., 2009; Launer, et al., 2011; McGill et al., 2007). The relatively large-scale Glycemic Effect in Diabetes Mellitus: Carvedilol-Metroprolol Comparison in Hypertensives (GEMINI) trial investigated effects of antihypertensive treatment on health outcomes. Here, no difference between two treatment arms in the change of self-reported cognitive deficits during a five-month follow-up was established, although incidence of hypoglycaemia over the same time period favoured one treatment arm (McGill, et al., 2007). These findings are supported by evidence from ACCORD-MIND and ADVANCE, where patients in intensive treatment groups (with higher incidence of hypoglycaemia) cognitively declined at similar rates over 40-month and five-year follow-up, respectively, compared with the respective standard treatment (de Galan, et al., 2009; Launer, et al., 2011), despite an initial non-significant trend for steeper decline in speed of processing in the intensive treatment group of ACCORD-MIND between baseline and 20-month interim assessment (Launer, et al., 2011). Specific study designs may account for the apparent failure to establish associations in these studies. In GEMINI, both the change in cognitive function and incidence of hypoglycaemia were ascertained using self-report, so that the findings may be unreliable. ADVANCE and ACCORD-MIND actually measured change in cognitive function, but both were trials on the effects of strict glycaemic control on health outcomes. Because improvements in glycaemic control may alleviate cognitive dysfunction in poorly-controlled type 2 diabetes (Ryan et al., 2006), it is plausible that detrimental effects of hypoglycaemia were counteracted by cognitive improvement associated with the assigned intervention.

In contrast to the non-significant evidence on cognitive decline in the dementia-free range, two prospective investigations based on hospital records revealed an increased risk of dementia over four years of follow-up in individuals who had been admitted for one or more episodes of SH during previous periods of between three and 18 years. Both studies further found dose-response relationships between the number of hospital admissions for SH and risk of dementia (Lin & Sheu, 2013; Whitmer, et al., 2009). A baseline history of hypoglycaemia evidenced in hospital records also doubled participants' risk of dementia over twelve years in the Health ABC Study. The finding persisted when adjusted for pre-dementia change in MMSE scores (Yaffe, et al., 2013), which may suggest a differential role of hypoglycaemia in dementia and cognitive decline. Contrasting these findings, a baseline history of SH failed to predict the five-year risk of conversion between normal functioning, reduced cognitive functioning and diagnosis with dementia in the Fremantle Diabetes Study (Bruce, et al., 2009).

Conclusions

Overall, cross-sectional evidence in type 2 diabetes suggests links between a history of hypoglycaemia and reduced level of cognitive function and the presence of dementia. These findings may mainly reflect associations in the cognition-hypoglycaemia direction, which to date several studies have pointed to. Although prospective investigations in type 2 diabetes have consistently failed to reveal detrimental effects of a history of hypoglycaemia on cognitive decline short of dementia, all were limited by specific study designs and are at odds with some evidence suggestive of an increased risk of dementia following the experience of hypoglycaemia. A prospective investigation of the relationship between hypoglycaemia and the spectrum of age-related cognitive decline in an observational setting in this type of patient is urgently needed.

3.2 Macrovascular disease

3.2.1 Symptomatic macrovascular disease

Symptomatic macrovascular disease and its prevalence in diabetes

Atherosclerosis results in macrovascular disease which affects the vasculature of the entire body. Cardiac macrovascular disease causes coronary heart disease (CHD) typically defined as myocardial infarction (MI) and/or presence of angina pectoris. Macrovascular disease of the brain may result in cerebral infarction, occurring either hemorrhagic or ischaemic. Intra-cerebral haemorrhage (ICH) results from a spontaneous rupture of a cerebral artery and causes accumulation of blood in brain tissue (Pleşea et al., 2005). In ischaemic strokes, which account for around 80% of all strokes, cerebral blood flow is interrupted due to the occlusion of a major artery (Sims & Muyderman, 2010). Transient ischemic attacks (TIA) are focal ischaemic strokes which remain episodic. Symptoms typically resolve within 24 hours, although for 30% to 50% of patients damage to the brain tissue is permanent (Easton et al., 2009; Poisson & Johnston, 2011), leading to the suggestion that a distinction between TIA and stroke may be futile (Easton, et al., 2009). Infarcts further occur in absence of symptoms as 'silent' microbleeds or 'silent' ischaemic strokes caused by leukoaraiosis, lacunar infarcts or territorial lesions (Gállego & Martínez-Vila, 2005). Silent infarcts are common, with prevalence of 20% even in healthy older adults (Vermeer, Longstreth, & Koudstaal, 2007). In 1998, for instance, around 11 million silent infarcts occurred in the USA, compared with only 200 000 to 500 000 TIAs (Easton, et al., 2009) and 770 000 strokes (Leary & Saver, 2003).

Macrovascular disease also affects the lower limbs. Around 16% of 55 year-olds have atherosclerotic occlusive disease in this part of the body. In around one third of these individuals, circulatory deficits become symptomatic in intermittent vascular claudication (PAD stage II) through discomfort, pain, cramping or aching in calves thighs or buttocks. Symptoms typically occur during walking exercise and are relieved by rest. In two thirds of individuals with PAD, the disease remains asymptomatic (stage I); around 1% are affected by limb ischaemia causing rest pain, tissue loss or gangrene (stage III-IV) (ADA, 2003; Waldstein et al., 2003). Symptomatic PAD has been linked to poor health outcomes, including a three-fold risk of cardiovascular events (Banerjee, Fowkes, & Rothwell, 2010).

Diabetes is an important risk factor for symptomatic macrovascular disease, including CHD, cerebral infarction and PAD (ADA, 2003; Carneiro, 2004; Hopkins & Williams, 1981). For instance, diabetes has been linked to a two-fold increased risk of brain infarction (Centers for Disease Control and Prevention, 2011), and has been estimated to account for around 12% of strokes (Luitse, Biessels, Rutten, & Kappelle, 2012). Consequently, prevalence of cerebral infarction (Mankovsky & Ziegler, 2004), CHD (Chaturvedi, 2007) and PAD (ADA, 2003) are all increased in diabetes and potentially also in pre-diabetes (Rydén et al., 2007). For instance, PAD may be prevalent in between 10% and 29% of patients with type 2 diabetes, compared with only 4% in the general population (ADA, 2003; Potier, Khalil, Mohammedi, & Roussel, 2011). Conversely, diabetes is also common in people with macrovascular disease (Mukherjee, 2009). This section of the chapter is concerned with the evidence of associations between symptomatic macrovascular disease and cognitive function in people with type 2 diabetes. Where the evidence is lacking, the literature on the general population is consulted, although potential differences between patients with diabetes and the general population in the relationship of macrovascular disease with cognition are not at the centre of the present research question.

Symptomatic macrovascular disease and cognition in type 2 diabetes

In order to ascertain the evidence on the topic from patients with type 2 diabetes, a systematic search of the literature was carried out for cross-sectional or prospective investigations of symptomatic macrovascular disease and cognition, which were performed in populations either consisting entirely of people with type 2 diabetes (or older adults with unspecified diabetes) or which reported analyses of diabetic subsamples from cohorts of the general population.

Search strategy

Titles, abstracts and keywords (.mp) of studies indexed by Medline and published between 1946 and the first week of May 2013 were searched for the terms "type 2 diabetes" OR "NIDDM" OR "non-insulin dependent", and "intelligence OR "cognitive function" OR "cognitive abilit*", as well as "macrovascular disease" OR "stroke" OR "cerebral infarct*" OR "TIA" OR "transient isch*mic attack" OR "angina" OR "myocardial infarction" OR "MI" OR "PAD" OR "intermittent claudication". The three separate searches were combined using AND. Additionally, reference lists of reviews and key articles were examined and experts in the field were consulted to identify additional publications.

Selection of studies

Studies were selected for inclusion if they fulfilled the following criteria: a) original studies b) cohort studies either exclusively of humans with type 2 diabetes, or unspecified diabetes and mean age >55 years, or studies reporting data for a diabetic subsample, c) ascertainment of symptomatic macrovascular disease, d) application of neuropsychological tests or clinical evaluation of dementia and e) reporting of data linking macrovascular disease and cognitive function. Although cerebral infarctions identified using brain imaging do not strictly imply *symptomatic* disease, studies which used this method were included with the aim to assess all of the evidence and because the causes of and damage associated with symptomatic and asymptomatic cerebral events are assumed to be identical. Analyses published on the basis of this thesis, as well as case studies and reviews of the literature were not included.

Data extraction

The titles and abstracts of all studies identified by the search were screened. For studies which appeared to meet the criteria, or where this could not be determined from the abstract alone, the full texts were obtained and reviewed for inclusion. Data from studies which were subsequently identified to meet the criteria were extracted, with focus on the following topics: study design, total number of participants, age at baseline (range or mean), measurement of cognitive function, the specific macrovascular disease under investigation and its measurement, main findings including effect sizes and p-values or confidence intervals where available, and potential limitations. Extracted data from all studies included in the review were tabulated.

Results

The search yielded 108 studies. Full texts were accessed for 25 studies, 10 of which failed to meet the inclusion criteria mainly because data on macrovascular disease, diabetes and cognition were reported, but associations amongst these variables were not explored. 15 studies were eventually deemed to meet the inclusion criteria and were included in the review (Figure 3.2). Their designs and main findings are summarised in Table 3.3 and Table 3.4.

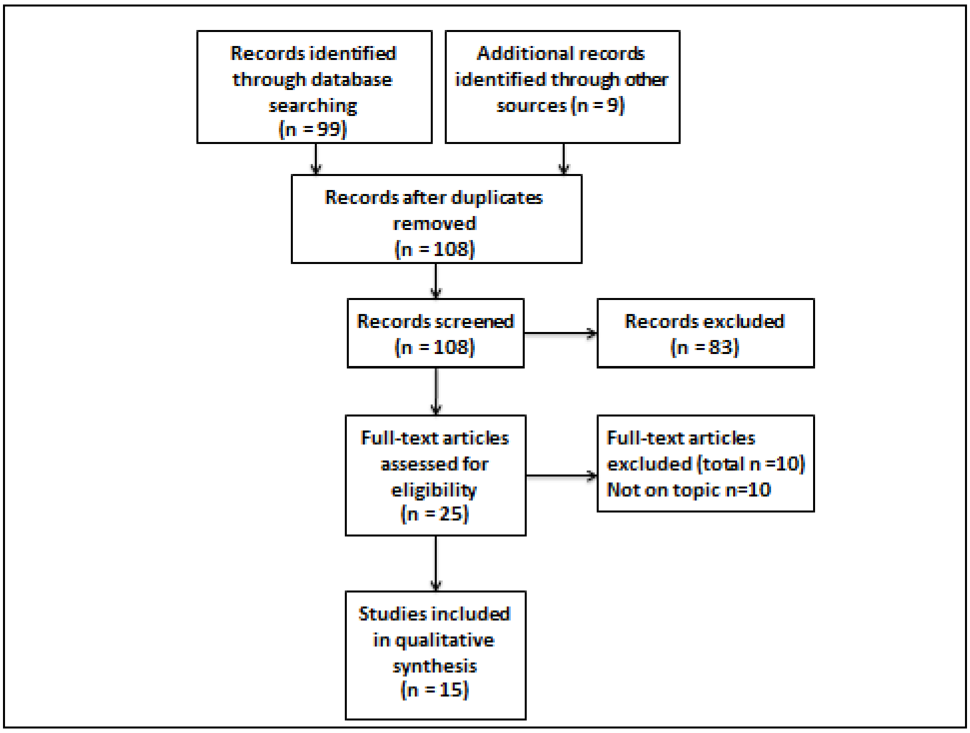

Figure 3.2: Systematic literature search on symptomatic macrovascular disease and cognition in type 2 diabetes

Table 3.3: Summary of studies identified in systematic search on symptomatic macrovascular disease and cognition in older adults with diabetes

	Sample	Design	Total n	Age at baseline	Macrovascular disease and its measurement	Measurement of cognitive function
Fushimi et al. (1996)	Patients with type 2 diabetes (80%) and non-diabetics, Japan	Cross-sectional	194	68 years	Lacunae on MRI	Cube-drawing test (spatial ability)
Bruce et al. (2001)	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Cross-sectional	63	≥70 years (mean 75 years)	Self-reported history of stroke	'Probable dementia' determined from combination of MMSE and IQCODE
Wu et al. (2003)	SALSA (Latinos; 33% with any type of diabetes), USA	2-year prospective	1789	>60 years (mean 71 years)	Self-reported history of stroke	MMSE; Delayed Word-List Recall. 'Major decline' defined as 10^{th} worst percentile change
Manschot et al. (2006)	Utrecht Diabetic Encephalopathy Study (113 type 2 diabetes patients, 51 controls; all free of dementia or disease affecting cognitive function), Netherlands	Cross-sectional	164	55-80 years (mean 66 years)	Self-reported history of MI, stroke or surgery/endovascular treatment for coronary, carotid or peripheral arterial disease. Lacunar/large cerebral infarcts on MRI	11 cognitive tests of domains memory, reasoning, processing speed, attention/executive function, visuospatial construction. Dutch version of NART
Manschot et al. (2007)	Utrecht Diabetic Encephalopathy Study (112 type 2 diabetes patients, 56 controls; all free of dementia or disease affecting cognitive function), Netherlands	Cross-sectional	168	55-80 years (mean 66 years)	1. 'Any PAD' defined as intermittent claudication on Rose questionnaire or self-reported history of surgery/endovascular treatment for peripheral or abdominal arterial disease 2. 'Ischaemic heart disease' defined as self-reported	Composite z score calculated from 11 cognitive tests of domains memory, reasoning, processing speed, attention/executive function, visuospatial construction. Dutch version of NART

					history of MI or surgery/endovascular treatment for CHD 3. 'Any vascular event' define as self-reported history of MI, stroke or surgery/endovascular treatment for coronary, carotid or peripheral arterial disease. 4. Lacunar/large cerebral infarcts on MRI	
Bruce et al. (2008) Diabetes Care	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Median 2-year prospective	205	≥70 years (mean 75 years)	1. 'Cerebrovascular disease' defined as self-reported history of stroke/ TIA supplemented by scrutiny of hospital records 2. 'CHD' defined as self-reported MI/ angina/ coronary artery bypass/ evidence of MI on EEG	1. MMSE, CDR, IQCODE 2. neuropsychological assessment. MCI defined as CDR=0.5 without dementia; 'cognitive decline' defined as conversion between normal cognition, MCI and dementia
Bruce et al. (2008) Diabetologia	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Median 8-year prospective	302	≥70 years (mean 76 years)	1. 'Cerebrovascular disease' defined as self-reported history of stroke/ TIA supplemented by scrutiny of hospital records 2. 'CHD' defined as self-reported MI/ angina/ coronary artery bypass/ evidence of MI on EEG	1. MMSE, CDR, IQCODE 2. neuropsychological assessment. MCI defined as CDR=0.5 without dementia; 'cognitive decline' defined as conversion between normal cognition, MCI and dementia
Cukiermann-Yaffe et al. (2009)	ACCORD-MIND (type 2 diabetes patients), North America	Cross-sectional	2977	>55 years (mean 63 years)	1. Stroke 2. 'CVD' defined as stroke/ MI/ angina with ischaemic	MMSE, RAVLT, DSC, Stroop

					changes/ coronary procedure	
De Galan et al. (2009)	ADVANCE (type 2 diabetes patients), Australia	Median 5-year prospective	11 140	>55 years (mean 66 years)	1. Stroke (fatal or non-fatal) 2. MI (fata or non-fatal) 3. Major coronary event (non-fatal MI or death from coronary disease) 4. Major CVD event (non-fatal MI/ non-fatal stroke/ cardiovascular death Macrovascular disease used as dependent variable.	3. MMSE 'Normal': >28 'Mild dysfunction': 24-27 'Severe dysfunction': <24 4. Neurospsychological assessment of 'severe' group
Ruis et al. (2009)	Anglo-Danish-Dutch Study of Intensive Treatment in People with Sceen Detected Diabetes in Primary Care (ADDITION) (183 patients with type 2 diabetes, 69 controls), RCT on multifactorial treatment, Netherlands	Cross-sectional	152	50-70 years (mean 63 years)	'Macrovascular disease' defined as history of MI, stroke, surgery/endovascular treatment for carotid, coronary or peripheral arterial disease (presumably self-report data)	1. 12 cognitive tests of domains reasoning, memory, processing speed, attention/executive function, visuoconstruction, language comprehension) 2. Dutch version of NART
Petrova et al. (2010)	Type 2 diabetes patients (all with memory complaint), Russia	Cross-sectional	113	40-70 years (mean 65 years)	'Cerebrovascular disease' ascertained using 'standard definitions'	MMSE, FAB, category fluency, Clock Drawing Test 'Cognitive impairment' defined as MCI-free but 'decline' on at least 1 cognitive test
Van Elderen et al. (2010)	Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) (89 patients with type 2 diabetes, 438 subjects without diabetes), RCT on effect of Pravastatin,	3-year prospective	527	70-82 years (mean 75 years)	Cerebral infarcts on MRI	Stroop, Picture Learning Test, Letter-Digit Coding Test

	Netherlands					
Imamine et al. (2011)	Type 2 diabetes patients, Japan	3-year prospective	67	>65 years (mean 75 years)	1. Presence of silent brain infarcts on MRI 2. 'Progression of silent brain infarcts' defined as increase by at least 1 between baseline and follow-up	MMSE, DSC, Stroop, Word Recall; calculation of change scores
Reijmer et al. (2011)	Utrecht Diabetic Encophalopathy Study (68 type 2 diabetes patients; 38 controls), Netherlands	4-year prospective	106	56-80 years (mean 65 years)	1. Cerebral infarcts on MRI 2. Self-reported 'cardiovascular disease' defined as MI or surgical or endovascular treatment of atherosclerotic arterial disease	11 cognitive tests of 3 domains (memory, processing speed, attention/executive function); cognitive decline calculated using composite regression-based index (RBI) compared with non-diabetic control group. 'Cognitive decline' based on RBI averaged from the 3 cognitive domains
Umemura et al. (2011)	Type 2 diabetes patients (dementia-free), Japan	6-year prospective	219	>45 years (mean 63 years)	Silent brain infarcts on MRI. 'Progression of infarcts' determined by blinded clinicians	MMSE, DSC, Word Recall, Stroop administered to 90 patients

SALSA, Sacramento Area Latino Study of Aging; MRI, magnetic resonance imaging; TIA, transient ischaemic attack; MI, myocardial infarction; CHD, coronary heart disease; MMSE, Mini Mental State Examination; CDR, Clinical Dementia Rating Scale; IQCODE, Informant Questionnaire on for Cognitive Decline in the Elderly; DSC, Digit Symbol Coding; RAVLT, Rey's Auditory Verbal Learning Test; FAB, Frontal Assessment Battery; NART, National Adult Reading Test; PAD, peripheral arterial disease; RCT, randomised controlled trial.

Table 3.4: Main findings from studies identified in systematic search

	Finding	Limitations
Fushimi et al. (1996)	Association between presence of lacunar infarcts and lower cognitive function in people with diabetes and non-diabetics (r=0.63, p=0.0001 in diabetic group)	
Bruce et al. (2001)	Prevalence of self-reported stroke similar in 'probable dementia' group and others (p=0.061)	Uncertain definition of 'probable dementia'
Wu et al. (2003)	1. Cross-sectional analysis of diabetes patients: association between history of stroke and lower level of cognitive function at year 2 (p<0.0001) 2. Prospective analysis of entire sample: association between history of stroke and 2-fold increased risk of 'major cognitive decline' on both cognitive tests (OR 2.01, 95% CI 1.22, 3.32, for MMSE and OR 1.95, 95% CI 1.22, 3.10, for word-list recall). Analyses adjusted for age, sex, education, hypertension.	Short follow-up
Manschot et al. (2006)	1. Diabetic group: association between infarcts on MRI (infarcts found in 19% of patients) and lower processing speed (β -0.77; p<0.05) (adjusted for age, sex, NART). No association with other cognitive domains. 2. Diabetic group: no association between 'any macrovascular disease' and cognitive test performance	
Manschot et al. (2007)	1. Diabetic group: 'any vascular event' associated with lower cognitive function (for z score β -0.25; p=0.01; for processing speed β -0.46, p=0.008; for memory β -0.23, p=0.01) (finding 'attenuated' on exclusion of patients with stroke; data not shown) 2. Diabetic group: association of infarct on MRI with lower cognitive function (for z score β -0.28; p=0.01; for processing speed β -0.77, p<0.001) (for z score, this did not survive adjustment for age, sex, NART) 3. Diabetic group: 'any PAD' entered in regression model (following adjustment for age, sex, NART), but apparently not retained	1. Findings for 'any PAD' not reported. 2. Unclear if 'attenuation' implies loss of statistical significance 3. Re-reports findings on cerebral infarcts previously described in Manschot et al. (2006)
Bruce et al. (2008) Diabetes Care	1. Whole sample: no association between CHD or cerebrovascular disease and risk of 'decline' 2. Similar results when analyses of cognitive decline restricted to individuals with 'normal cognition' at baseline	
Bruce et al. (2008) Diabetologia	1. Cross-sectional: prevalence of stroke increased in MCI and dementia compared with unimpaired patients (unadjusted OR 3.18, p<0.001 for MCI/ dementia). Prevalence of CHD equivalent in all 3 groups (p=0.14) 2. Prospective: Higher prevalence of cerebrovascular disease at baseline predicted poorer cognitive outcome at year 8 (prevalence 7% in 'normal', 10% in MCI	Did not explore change in cognitive function between baseline and year 8

	and 25% in dementia group; p=0.008 across groups); CHD unrelated to cognition at year 8 (p=0.15)	
Cukiermann - Yaffe et al. (2009)	1. Association between stroke and lower scores on 4 cognitive tests (p<0.0001 to p<0.05). For instance, subjects with stroke had 8-point lower DSC scores and 0.6 unit lower MMSE scores 2. Association between CVD (minus stroke) and lower RAVLT but higher MMSE (both p<0.05)(no association with DSC, Stroop)	
De Galan et al. (2009)	1. Cross-sectional: Association between history of stroke and poorer cognitive group (history of stroke prevalent in 22% of individuals with dementia, 12% of individuals with 'mild dysfunction' and 8% of others, p<0.001 across groups); no association between MI and cognitive group (p=0.61) 2. Prospective: Baseline 'mild cognitive dysfunction' and 'severe cognitive dysfunction' both associated with increased risk of major CVD event, stroke and major coronary event (e.g. with 31% and 70% increased risk of major coronary event with p=0.004 and p=0.020 respectively when adjusted for age, sex, education, treatment)	Did not report outcome of neuropsychological assessment of 'severe dysfunction' group so prevalence of dementia is unclear
Ruis et al. (2009)	1. Diabetic group: association between macrovascular disease and lower processing speed (standardised β -0.17; p<0.01) (adjusted for age, sex, NART). No association with memory domain.	1. Associations with other cognitive domains were not explored. 2. Unclear *which* of the macrovascular disease variables contributed to finding
Petrova et al. (2010)	Increased prevalence of impairment on MMSE, FAB, category fluency in patients with cerebrovascular disease (23%, 63% and 24% respectively) compared with cerebrovascular disease-free individuals (8%, 21% and 8% respectively) (p<0.0001 to p=0.002). No group differences in impairment on Clock Drawing Test (70% versus 92%; p=0.12)	Unclear definition of 'cerebrovascular disease', 'impairment' and 'decline' (study is cross-sectional)
Van Elderen et al. (2010)	Diabetic group: no association between baseline infarcts on MRI and change in cognitive function	Data not shown
Imamine et al. (2011)	1. Cross-sectional: association between baseline number of silent infarcts and baseline MMSE, Word Recall, DSC, Stroop (r=-0.25 on MMSE, p<0.05 to r=-54, p<0.001 on Stroop), and follow-up Word Recall, DSC, Stroop (r=-0.37, p<0.005 on Word Recall to r=-0.48, p<0.001 on Stroop) 2. Prospective: trend for association between baseline number of silent infarcts and DSC change score (r=-0.21, p<0.10) but not to change in other cognitive tests. Progression of silent infarcts unrelated to any cognitive outcome. All analyses adjusted for age, sex, education, treatment, vascular risk factors)	
Reijmer et al. (2011)	1. Diabetic group: no association between baseline infarcts on MRI and 'cognitive decline' (95% CI	

	including 0) 2. Diabetic group: no association between baseline CVD and 'cognitive decline' (95% CI including 0)	
Umemura et al. (2011)	Individuals with 6-year progression of silent brain infarcts had lower level of DSC and Stroop compared with remaining participants (both Cohen's *d*=3.97, p<0.01) (no association with MMSE or Word Recall)	Cognitive function assessed at year 6 only, and only in subsample

MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; DSC, Digit Symbol Coding; FAB, Frontal Assessment Battery; RAVLT, Rey's Auditory Verbal Learning Test; CHD, coronary heart disease; CVD, cardiovascular disease; CI, confidence interval; NART, National Adult Reading Test.

Overview of included studies

The 15 studies reported data from 11 populations. All studies except 3, which were randomised controlled trials on the effects of intensified glycaemic control or lipid-lowering drugs, had observational study designs. All reported data on cerebral infarctions; of these 7 had cross-sectional designs and 8 were prospective. Only 5 studies (reporting results from 4 populations) investigated CHD. Of these, 3 had prospective designs and 2 were cross-sectional. One study on symptomatic PAD and cognitive function was identified. The total number of participants included in the review were 18 186. Eleven studies were on patients with type 2 diabetes; 4 included older patients with any type of diabetes. All studies included males and females. Mean age of participants ranged between 63 and 76 years. Two studies investigated clinical diagnosis with dementia, whereas all of the remaining studies administered neuropsychological tests or cognitive screening instruments. One of these 13 studies additionally included clinical interview of a 'severe cognitive dysfunction' group, although the outcome of the interview was not reported. With this overall heterogeneity between the studies, meta-analysis to derive overall effect sizes of associations between macrovascular disease and cognitive outcome was deemed not appropriate.

Findings from included studies

Coronary heart disease (CHD) and cognition

Five studies which examined the association between CHD and cognitive function in people with type 2 diabetes were identified by the literature search (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008; Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008; Cukierman-Yaffe, et al., 2009; de Galan, et al., 2009; Reijmer, et al., 2011). In cross-sectional analyses of ACCORD-MIND, CHD was associated with lower memory performance, but was unrelated to processing speed or executive function measures. Unexpectedly, individuals with CHD had significantly higher MMSE scores compared with CHD-free subjects (Cukierman-Yaffe, et al., 2009). All of the findings from the remaining cross-sectional studies identified in the search were non-significant. A baseline history of CHD was unrelated to the presence of reduced late-life cognitive function that was short of dementia in ADVANCE (de Galan, et al., 2009), and in the Fremantle Diabetes Study to either reduced cognitive function or to presence of diagnosed dementia. In the latter, baseline CHD was also unrelated to the cognitive outcome eight years later, although the cognitive trajectories between baseline and follow-up were not explored (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008). The role of CHD in the reduced cognitive function observed in patients with 'any vascular event' in the Utrecht Diabetic Encephalopathy Study is unclear (Manschot et al., 2007).

Results from the prospective analyses also consistently revealed non-significant associations between CHD and subsequent cognitive decline or risk of dementia. In a Dutch cohort, a history of MI or surgical/endovascular treatment of atherosclerotic arterial disease did not predict the risk of accelerated four-year cognitive decline (identified on the basis of eleven age-sensitive neuropsychological tests) relative to a non-diabetic control group (Reijmer, et al., 2011). Presence of CHD was also unrelated to the two-year risk of downward conversion between normal functioning, reduced cognitive function short of dementia and frank dementia, or to risk of any cognitive impairment (reduced cognitive function/dementia) in the Fremantle Diabetes Study (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008).

The only significant prospective findings have been reported in the cognition-CHD direction. In ADVANCE, the baseline presence of 'mild cognitive dysfunction' as well as 'severe cognitive dysfunction' (which may or may not have included individuals with frank dementia) increased the risk of a major coronary event (non-fatal MI or death from coronary disease) during five years of follow-up by 31% and 70% respectively after multivariable adjustment aimed at reducing the potential influence by confounding factors linked to both cognitive function and CHD (de Galan, et al., 2009). Overall, this evidence suggests that CHD may not play an important role in the determination of cognitive outcome in patients with type 2 diabetes. Due to the currently limited evidence of associations in the cognition-CHD direction to a single study (which may or may not reflect causality), the role of cognitive function in the determination of risk of CHD in this patient is yet unclear.

Cerebral infarcts and cognition

Eleven studies were identified which investigated symptomatic or asymptomatic cerebral infarction and cognition in people with type 2 diabetes. In terms of cross-sectional analyses, an early study of 56 older diabetes patients set in Japan revealed associations of large effect size between evidence of infarction on MRI and a lower spatial ability (Fushimi, Inoue, Yamada, Udaka, & Kameyama, 1996). In a more recent but similarly small Japanese study of stroke-free diabetes patients, a higher number of silent brain infarcts on MRI was linked to lower MMSE, memory, speed of processing and executive function, with relatively largest effect sizes for the latter (Imamine et al., 2011). In the diabetic subsample of the Utrecht Diabetic Encephalopathy Study, individuals with evidence of brain infarcts on MRI also had lower processing speed and global ability measured by scores on a composite measure derived from twelve cognitive tests. Additionally, links between a self-reported history of 'any vascular event' and lower global ability, processing speed and memory were attenuated following exclusion of patients with stroke, demonstrating some contribution of stroke to the reduced cognitive function in this group (Manschot, et al., 2007). Individuals with self-reported history of stroke also had lower MMSE scores, as well as processing speed, executive function and verbal memory in ACCORD-MIND. For instance, stroke was linked to a 0.6-point lower score on the MMSE (maximum 30 points) (Cukierman-Yaffe, et al., 2009). A history of stroke was more prevalent in individuals with reduced level of cognitive function in ADVANCE. Specifically, 22% of patients with ‘severe cognitive dysfunction’ (who may or may not have suffered from dementia) had a history of stroke, compared with 12% of ‘mildly impaired’ individuals and 8% of the remaining sample (de Galan, et al., 2009). Similarly, a smaller study performed in diabetes patients with memory complaint revealed increased prevalence of ‘impairment’ on the MMSE and tests of executive function in patients with a history of cerebrovascular disease. For instance, 23% of individuals with cerebrovascular disease were impaired on the MMSE compared with 8% of patients free of cerebrovascular disease, although the specific definitions of ‘impairment’ were unclear (Petrova, et al., 2010). Patients with diabetes and a history of stroke further had lower cognitive function compared with stroke-free diabetes patients in a diabetic subsample of the SALSA cohort of older Latinos in the US (J. H. Wu, et al., 2003).

Progression of silent brain infarcts during six years predicted lower processing speed and executive function at follow-up in a study of 219 middle-aged to older type 2 diabetes patients, although results for other cognitive tests were non-significant (Umemura et al., 2011). Null results were also reported for links between a history of stroke and presence of reduced cognitive function identified on the basis of cognitive screening instruments in the Fremantle Diabetes Study (Bruce, et al., 2001). Yet, when screening was followed up by clinical interview in the same cohort, individuals with reduced cognitive function short of dementia and those with dementia diagnosis were found to be more likely to have had a prior history of cerebrovascular disease (stroke and/or TIA) compared with cognitively ‘normal’ individuals. In unadjusted analyses, stroke alone was associated with a three-fold increased risk of presence of reduced cognitive function or dementia. Baseline cerebrovascular disease further predicted poorer cognitive outcome (presence of impairment short of dementia or dementia) at year 8 in the same study, although associations with eight-year cognitive decline were not explored (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008).

Findings from the diabetic subsample of the Utrecht Diabetic Encephalopathy Study of associations between infarction on MRI and lower global cognitive function and processing speed (Manschot et al., 2006) (but not global ability (Manschot, et al., 2007)) survived adjustment for an estimate of pre-morbid ability, suggesting that individuals with presence of infarction experienced accelerated decline between pre-morbid speed in young adulthood and late-life post-stroke speed. This result was subsequently replicated in another Dutch cohort, although here all (presumably self-reported) symptomatic macrovascular disease variables were grouped together. Consequently, the contribution of individual factors, such as stroke, to associations of macrovascular disease with a steeper estimated lifetime decline in processing speed remains unclear (Ruis, et al., 2009).

In terms of prospective analyses, a higher number of infarcts on MRI at baseline predicted a trend short of statistical significance for steeper three-year decline in processing speed in the small Japanese study, although the change in other cognitive tests was entirely unrelated to baseline infarction and to the progression of silent brain infarcts over this time period (Imamine, et al., 2011). In an analysis of the entire SALSA cohort (a substantial proportion of which had diabetes), baseline history of stroke also predicted a two-fold increased risk of ‘decline’ on the MMSE and on a test of verbal memory, although the authors did not stratify by diabetes status (J. H. Wu, et al., 2003). Evidence of cerebral infarction on MRI was unrelated to the four-year cognitive decline short of dementia in the diabetes patients relative to non-diabetic controls in the follow-up of the Utrecht Diabetic Encephalopathy Study (Reijmer, et al., 2011) or to the three-year decline short of dementia in a diabetic subsample of a Dutch randomised controlled trial on the effects of cholesterol-lowering drugs (van Elderen, et al., 2010). In the Fremantle Diabetes Study, baseline cerebrovascular disease was unrelated to conversion between ‘normal’ functioning, impairment short of dementia and frank dementia over two years of follow-up, both when the entire sample was considered and when analyses were restricted to individuals with initial ‘normal’ cognition at baseline (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008).

The ADVANCE trial was identified as the only study of associations in the cognition-infarction direction in people with diabetes. After multivariable adjustment, individuals with 'mildly reduced' cognitive function at baseline were at 34% increased risk of a major stroke during the subsequent five years compared with individuals with higher cognitive function; patients with 'severe cognitive dysfunction' were even at 71% increased risk (de Galan, et al., 2009).

Overall, the review of the literature established that a prior history of cerebral infarction is relatively consistently linked to a poorer cognitive function (most frequently to poorer executive function and processing speed), and has increased prevalence in people with dementia in older adults with diabetes. The prospective evidence is less consistent, but points to potential bidirectional associations.

Symptomatic peripheral arterial disease (PAD) and cognition

In the only study identified in the systematic search with data on symptomatic PAD and cognitive function in patients with diabetes, PAD was entered into a regression model following adjustment for age, sex and estimated pre-morbid ability, but was not retained in the model. This suggests that PAD may not have been related to the estimated lifetime cognitive change in the sample, although neither statistical results nor findings from unadjusted analyses were reported by the authors (Manschot, et al., 2007).

Comparison of findings from the systematic search with evidence in the general population

Because the evidence on symptomatic macrovascular disease and cognition in people with type 2 diabetes is relatively limited, the findings from the systematic review were put in context with the literature on the general population with the aim to offer an overall picture of the evidence and to address any inconsistency.

CHD and cognition

The systematic review showed that the presence of CHD appears to be relatively unrelated to cognitive function in people with type 2 diabetes. Yet, in the general population, several studies have revealed cross-sectional associations between a presence of CHD and a low level of cognitive function short of dementia (Gharacholou et al., 2011; Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007; Roberts et al., 2008; Singh-Manoux, Britton, & Marmot, 2003). This contrasting evidence may be due to the limited literature available on people with diabetes. Alternatively, it may be the case that the health of people with diabetes is monitored more carefully compared with the general population, so that CHD is detected at earlier stages. The null findings for associations with cognitive function in diabetes could then arise due to a relatively lower severity of CHD in these patients; CHD patients in the general population may be affected more severely at the point of diagnosis so that any subtle associations between the presence of CHD and lower cognitive function may be more easily detectable in this group. Some of the studies of people with diabetes also had categorised their samples according to cognitive function (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008; de Galan, et al., 2009), and so may have had reduced statistical power to detect associations when compared with the studies of the general population, many of which treated cognitive function as a continuous measure (e.g., (Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007)).

Yet, in some support of the null findings from the two prospective investigations in people with type 2 diabetes, prospective investigations associating CHD with cognitive decline in the general population have produced mixed results (Almeida, Beer, Lautenschlager, Arnola, et al., 2012; Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007; Verhaegen, Borchelt, & Smith, 2003). For instance, one study which used EEG to identify CHD reported an association between a baseline history of MI and increased risk of dementia over up to 15 years, but the finding was restricted to males and to previously *unrecognised* MI (Ikram et al., 2008).

Studies of interventions may help to resolve the contrasting evidence from cross-sectional and prospective studies in the general population. In line with a potential causal relationship between cardiovascular disease and cognition, cardiac transplantation and pacemaker fitting appear to improve cognitive function, and to alter cerebral blood flow and the P300 component evoked by cognitive processing

(Bornstein, Starling, Myerowitz, & Haas, 1995; Grimm et al., 1996; Koide, Kobayaski, Kitani, Tsunematsum, & Nakazawa, 1994). Yet, non-significant results have also been reported (Rockwood, Dobbs, Rule, Howlett, & Black, 1992; Schall, Petrucci, Brozena, Cavarocchi, & Jessup, 1989) and a number of factors unrelated to cardiac surgery itself, such as improved quality of life, could contribute to the observations.

Directly contrasting these beneficial effects of the aforementioned types of cardiac surgery on cognitive function, coronary artery bypass grafting (CABG) has been linked to post-surgery cognitive impairment in 30% to 80% of patients (Symes, Maruff, Ajani, & Currie, 2000). Importantly, diabetes appears to exacerbate this risk. In one Japanese study, pre- to post-CABG declines of >1 standard deviation on at least two of six cognitive tests over seven days was more common in patients with diabetes than in controls (Kadoi, Saito, Fujita, & Goto, 2005). Similar results were reported in another study of 127 older adults undergoing CABG. Patients with diabetes (but not non-diabetic patients) cognitively declined between pre-surgery assessment and assessments at one month and one year (Selnes et al., 1999).

Overall, the balance of evidence suggests that, whereas CHD may be linked to reduced cognitive function, it is unclear whether this may be due to associations in the cognition-CHD direction or confounding by other risk factors associated both with CHD and with cognition. Coronary heart disease does not appear to predict cognitive decrements either in the general population or in people with diabetes. Despite some evidence which suggests that diabetes may exacerbate post-cardiac-surgery cognitive deficits, the increased prevalence of CHD in type 2 diabetes is presumably unable to account for the increased risk of cognitive decline in people with diabetes.

Cerebral infarcts and cognition

Similar to the findings of studies carried out in people with diabetes as described in the systematic review, a self-reported history of cerebral infarction or imaging data consistent with infarction have frequently been linked to a lower scores on tests of fluid-type cognitive ability or screening instruments for dementia in the general population (Arvanitakis, et al., 2006; Breteler, Claus, Grobbee, & Hofman, 1994; Maeshima et al., 2002; T. R. Price et al., 1997). For instance, in a Scottish cohort, a baseline history of stroke was associated with poorer executive function and verbal memory (Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007). Silent or symptomatic infarctions also increase the risk of 'post-stroke dementia', and particularly vascular dementia (Pendlebury & Rothwell, 2009; Vermeer et al., 2003). In one relatively large Italian study, stroke increased the likelihood of either clinically diagnosed reduced cognitive function short of dementia or diagnosis with dementia comparable to an additional ten years of age (De Ronchi et al., 2007). As many as 72% and 56% of patients with vascular dementia had a history of stroke and TIA respectively compared with prevalence of 10% and 14% of control subjects in a recent Finnish study of relatively old individuals (Hiltunen et al., 2013).

The systematic review identified mixed prospective evidence of cerebral infarcts and cognitive function in people with diabetes, with four of five studies reporting non-significant associations. The literature on the general population appears more consistent. In one Dutch study of older adults, a self-reported history of cerebrovascular disease at baseline was linked to almost five-fold increased the risk of 'steep' decline on the MMSE over three years (Kalmijn, Feskens, Launer, & Kromhout, 1996). Baseline stroke also predicted accelerated decline on the MMSE during seven years in the Cardiovascular Health Study (Haan, et al., 1999). Evidence of cerebral infarction on MRI as well as a self-reported or clinically evidenced history of stroke have further been found to predict steeper four- to seven-year decline in older age on more detailed neuropsychological tests in several cohorts (Haan, et al., 1999; Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007; Vermeer, et al., 2003).

Similar to the findings of ADVANCE (de Galan, et al., 2009), studies in general older populations have also implicated a lower level of cognitive function with increased future risk of cerebral infarction (M. F. Elias et al., 2004). A review of the literature determined that pre-stroke dementia may be present in as many as 9% to 14% of patients with stroke (Pendlebury & Rothwell, 2009). Yet, because dementia occurs in around 20% of stroke patients three to six months after the event even when excluding people with a pre-stroke history of dementia (Pendlebury & Rothwell, 2009), some causation in the infarct-cognition direction is supported.

Overall, the findings from prospective investigations suggest that, as well as potentially reflecting confounding by other risk factors, associations of cerebral infarction and cognitive decline may provide evidence that cerebral infarction negatively impacts cognitive function in people with type 2 diabetes and in the general population. The relatively stronger evidence in the latter compared with the former could be due to the relatively small number of prospective studies carried out in patients with diabetes or lower statistical power due to relatively smaller sample sizes. Differences in risk of incident infarction over the course of prospective investigations appear less likely to account for the observation, given that people with type 2 diabetes are at *increased* risk of stroke (Centers for Disease Control and Prevention, 2011) despite apparently showing less evidence of stroke associations with cognitive decline compared with the general population.

Finally, some evidence suggests links between a lower cognitive ability and an increased risk of cerebral infarction in people with diabetes as well as in the general population. Thus, the relationship between cerebral infarction and cognition appears to be largely similar in people with diabetes and the general older population, and may be bidirectional. Considering that diabetes is a major risk factor for post-stroke dementia and reduced rate of cognitive recovery following stroke (Erkinjuntti, 2007; Pendlebury, 2009), it is plausible that cerebral infarction may play an important role in the increased risk of cognitive decline in people with diabetes.

Symptomatic peripheral arterial disease (PAD) and cognition

Although the literature on symptomatic PAD and cognition in people with type 2 diabetes is limited to a single investigation, several studies carried out in the general population report significant findings. In the Helsinki Aging Study of 650 old-old individuals, those with intermittent claudication had around two-point lower mean MMSE scores (maximum 30 points) compared with claudication-free individuals (Tilvis, et al., 2004). Presence of claudication was also linked to lower level of executive function, memory and reasoning with small to medium effect sizes in 5292 middle-aged civil servants of the Whitehall II study (Singh-Manoux, et al., 2003), although another study of 1500 middle-aged to older men established similar cognitive function in individuals with intermittent claudication and a control group free of any vascular disease (Elwood, Pickering, Bayer, & Gallacher, 2002). A recent analysis reported significant differences in prevalence of PAD across groups between cognitively healthy individuals (11%), patients with Alzheimer's Disease (2%), with vascular dementia (19%) and those with dementia with Lewy bodies (7%), although 'PAD' was not specified and pairwise group comparisons were not applied (Hiltunen, et al., 2013). Cross-sectional associations are restricted by observations of lower estimated pre-morbid ability in individuals with intermittent claudication (Singh-Manoux, et al., 2003), but are supported by prospective evidence. In the Helsinki Aging Study, baseline claudication predicted a 70% increased risk of 'steep' (≥4-point) decline on the MMSE and/or an increase in the severity of dementia defined using the Clinical Dementia Rating scale during one-year follow-up, although it was not linked to risk of these outcomes during five-year follow-up of the same study (Tilvis, et al., 2004), or to incidence of clinically diagnosed dementia during six years in a cohort of 201 old-old community-dwelling Australians (Piguet et al., 2003). Here, individuals with and those without baseline claudication also declined at similar rates on the MMSE over the course of the study. Overall, the evidence is relatively limited even in the general population and appears inconsistent. The contribution of intermittent claudication to cognition may become clearer in the consideration of the literature on *asymptomatic* PAD and cognition, which is described in section 3.2.2.

Summary of the evidence on associations of symptomatic macrovascular disease with cognition

In both people with type 2 diabetes and in the general older population, the presence of macrovascular disease manifesting in symptoms in various areas of the body has been associated with a reduced level of cognitive function including an increased likelihood of dementia. However, the direction of the associations and the role of potential clinical and demographic confounders, as well as pre-morbid ability, in any cross-sectional findings are unclear. For CHD and PAD, the evidence from prospective analyses appears to be mixed in both people with type 2 diabetes and in the general population. Compared with this, the prospective evidence for links of infarction occurring directly in the brain, i.e. the 'substrate' of cognition, with cognitive decline is more consistent at least in the general population. This type of investigation has produced mixed results in studies of people with type 2 diabetes, but this may be due to fewer studies focusing on this type of patient.

3.2.2 Potentially asymptomatic macrovascular disease

Systemic atherosclerotic changes may not always result in symptoms, but may become apparent in, e.g., asymptomatic cardiac stress, asymptomatic PAD and in increased carotid intima-media thickness (cIMT). Such subclinical markers may be useful in the analysis of associations of macrovascular disease with cognition. Due to higher prevalence of asymptomatic than symptomatic disease, and because it does not require categorisation into binary 'disease' versus 'no disease' groups, statistical power to detect associations is possibly higher than is found with studies of symptomatic disease. This section summarises the evidence on links between these markers of macrovascular disease, which may or may not have reached the point of clinical expression, and cognition.

<u>Natriuretic peptides</u>

Congestive heart failure (CHF), diabetes and cognition

CHF is a progressive condition and an end-stage of heart disease (Kannel, Hjortland, & Castelli, 1974). In addition to anecdotal evidence (Rogers et al., 2000), a number of quantitative studies have established links between symptomatic CHF or CHF severity and lower cognitive function (Almeida & Tamai, 2001; Antonelli Incalzi et al., 2003; Gorkin et al., 1993; Trojano et al., 2003; Zuccala et al., 1997), as well as reduced cerebral blood flow (T. C. T. F. Alves et al., 2005). A review of the literature notes that especially attention and memory may be compromised (Bennett & Sauvé, 2003). Several prospective investigations have also revealed associations with a steeper decline on detailed neuropsychological tests of fluid-type ability (Almeida, Beer, Lautenschlager, Arnolda, et al., 2012; Hjelm et al., 2012). In its most severe form, CHF-associated cognitive impairment may result in 'cardiogenic dementia'.

Some degree of impairment appears to be prevalent in 30 to 80% of CHF patients. This is independent of individual differences in education (i.e., pre-morbid ability) or co-morbidities (Bennett & Sauvé, 2003), although one recent report suggested that diabetes may exacerbate CHF associations with poorer cognitive function (Alosco et al., 2012a). This is important, because prevalence of CHF is increased in people with diabetes (Schainberg, Ribeiro-Oliveira, & Ribeiro, 2010), and diabetes is common in people with CHF. A systematic review found that 47% of US heart failure patients suffer from diabetes (Kamalesh & Nair, 2005).

The inactive metabolite N-terminal pro-brain natriuretic peptide (NT-proBNP) is a biomarker of the cardiac stress caused by CHF and ventricular dysfunction in general older, in diabetic (J. Y. Kim et al., 2007) and in other patient populations (Ambrosi, Singerorzan, Oddoze, Argues, & Heim, 2010). Pre-proBNP is synthesised in the ventricles, and particularly the left ventricle, by the cardiac muscle and is modified into the pro-hormone proBNP, which is released in short bursts as a result of myocyte stretch. Subsequently, proBNP is cleaved into the 76 amino-acid peptide NT-proBNP and the 32 amino-acid peptide BNP (Ashley, Galla, & Nicholls, 2008). Consequently, NT-proBNP and BNP have been found to correlate (Castleberry et al., 2010); plasma BNP>100 ng/l indicate established heart failure in clinical practice (Kondziella, Goethlin, Fu, Zetterberg, & Wallin, 2009).

Raised natriuretic peptides have been linked to male sex, poor renal function and inflammation (Resl, Hülsmann, Pacher, & Clodi, 2009), and to increased risk of symptomatic macrovascular disease (Di Angelantonio et al., 2009) and mortality (Barents et al., 2008). Similar associations have been reported in patients with type 2 diabetes (Gaede, Hildebrandt, Hess, Parving, & Pederson, 2005; Hamano, Abe, Komi, & Koboyashi, 2010; Vergès et al., 2005), who (consistent with increased prevalence of CHF) tend to have raised NT-proBNP (Andersen, Poulsen, Knudsen, Heickendorff, & Mogensen, 2005; Magnusson et al., 2004). Even in non-diabetic individuals, HbA1c may correlate with NT-proBNP (Clodi et al., 2009) and its correlate BNP (Suskin et al., 2000) (although one study implicated raised NT-proBNP as protective of diabetes risk (Everett et al., 2011)). The mechanisms linking diabetes with cardiac stress may involve hyperglycaemia and hyperinsulinemia, which contribute to increased left ventricular mass and reduced cardiac output. Hyperglycaemia further causes endothelial apoptosis and inflammation (which contributes to atherosclerosis), as well as oxidative stress and alteration in the nitric oxide metabolism, damaging the myocardium. Finally, raised insulin may activate the sympathetic nervous system and thereby affect heart function (Schainberg, et al., 2010).

Natriuretic peptides and cognition

To ascertain the observational evidence from cross-sectional or prospective studies on associations between NT-proBNP or BNP and cognition in type 2 diabetes, a systematic search was performed. The atrial natriuretic peptide (ANP) or its inactive N-terminal form (NT-proANP) are also part of the natriuretic peptide family, but are not considered here. In the discussion of the findings presented in this thesis, the term 'natriuretic peptide' therefore refers specifically to NT-proBNP and BNP.

Search strategy

Titles, abstracts and keywords (.mp) of studies indexed in Medline and published between 1946 and the first week of May 2013 were searched for the terms "type 2 diabetes" OR "non-insulin-dependent" OR "NIDDM", "natriuretic peptid*" OR "*BNP", and "cognitive function" OR "intelligence" OR "cognitive abilit*" were combined using AND. Additionally, other databases were searched, and reference lists of reviews and selected original articles were scanned. Experts in the field were consulted to identify additional articles. Because this search yielded no results (with exception of analyses published on the basis of this thesis), the search was widened to any studies of humans (diabetic or non-diabetic). The final search terms were "natriuretic peptid*" OR "*BNP", and "cognitive function" OR "intelligence" OR "cognitive abilit*", combined by an AND operator. Other databases were searched, and reference lists of reviews and selected original articles were scanned and experts in the field were consulted.

Selection of studies

Studies were selected for inclusion the following criteria were met: a) original studies, b) studies of any humans (either from the general population or from patient populations), c) measurement of BNP and/or NT-proBNP, d) application of neuropsychological test, brain imaging or clinical evaluation of dementia and e) reporting of data linking natriuretic peptides with cognitive function and/or brain imaging findings. Case studies, reviews of the literature and analyses published on the basis of this thesis were not included.

Data extraction

All titles and abstracts identified by the search were screened, and full texts were obtained for studies which appeared to meet the inclusion criteria or where this was uncertain. Data from studies which were found to meet the criteria were extracted, with focus on study design, total number of participants, age at baseline (range or mean), the specific natriuretic peptide (NT-proBNP and/or BNP), measurement of cognitive function, main findings including effect sizes and p-values or confidence intervals where available, and potential limitations. Although not part of the present research question, any available data on diabetes were also extracted. All data were tabulated.

Results

Following removal of duplicates, the search resulted in 50 articles. Full texts were accessed for 24 articles, and 19 of these were eventually found to meet the criteria for inclusion (Figure 3.3). The excluded 31 studies reported data from animal studies, had qualitative designs, were case studies or reported data presented in this thesis. The study designs and main findings of the 19 included studies are summarised in Tables 3.5 and 3.6.

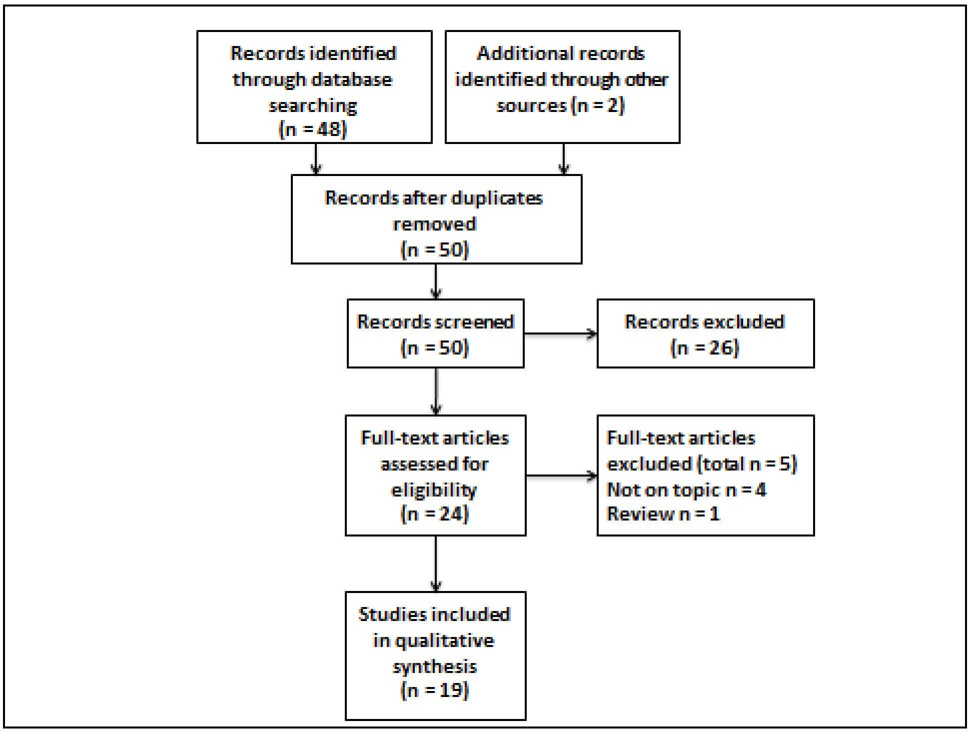

Figure 3.3: Systematic literature search on natriuretic peptides and cognition

Table 3.5: Summary of studies identified in systematic search on natriuretic peptides and cognition in studies of the general or any patient population

	Sample	Design	Total n	Age at baseline	Natriuretic peptide	Measurement of cognitive function
Gunstad et al. (2006)	Adults with CVD (dementia-free), USA	Cross-sectional	56	55-85 years (mean 70 years)	BNP	DRS
Mauro et al. (2007)	Patients with CHF, Italy	Cross-sectional	72	Mean 66 years	BNP	MMSE, Corsi-Block-Tapping
Nilsson et al. (2008)	Consecutively enrolled psychogeriatric patients (47% have dementia; all free of heart failure), Sweden	Cross-sectional	451 (392 with imaging data)	Median 75 years	NT-proBNP (cut-off applied to exclude patients with heart failure)	1. CT scans (categorised as ‘infarctions and white matter lesion’ versus ‘white matter lesion only’ versus normal) 2. Clinical diagnosis with dementia or reduced cognitive function short of dementia
Suwa & Ito (2008)	Patients with CVD, Japan	Cross-sectional	81	≥65 years	BNP	MMSE (‘cognitive impairment’ defined as MMSE<24)
Daniels et al. (2009)	Rancho Bernardo Study, USA	Cross-sectional	950	≥60 years (mean 77 years)	NT-proBNP	MMSE, TMT-B, Category Fluency
Kondziella et al. (2009)	Patients with VaD, patients with AD and controls, Sweden	Cross-sectional	49	Mean 70 years	BNP	Diagnosed dementia
Naito et al. (2009)	Patients with dementia (free of cardiovascular disease), Japan	Cross-sectional	42	Mean 74 years	BNP	HDS-R
Vaes et al. (2009)	Leiden 85+ Study, Netherlands	Cross-sectional	274	90 years	NT-proBNP	MMSE (‘poor cognitive function’ defined as MMSE<19)
Kerola et al. (2010)	Kuopio 75+(free of dementia at baseline), Finland	5-year prospective	464	≥75 years (mean 80 years)	BNP	Diagnosed dementia; MMSE

Nilsson et al. (2010)	Consecutively enrolled psychogeriatric patients (47% have dementia), Sweden	Cross-sectional	452	Median 75 years	NT-proBNP	MMSE; CT scan
McDonagh et al. (2010)	Patients undergoing surgery (free of mental illness, stroke, alcoholism), USA	Total 1-year prospective	394	>55 years (mean 68 years)	BNP	Randt Memory Test, Digit Span, Visual Reproduction, DSC, TMT-B. 'Post-operative cognitive dysfunction' (POCD) defined as >1SD decrease in scores on at least 1 of the 4 cognitive domains
Daniels et al. (2011)	Rancho Bernardo Study, USA	Cross-sectional	950	≥60 years (mean 77 years)	NT-proBNP	MMSE, TMT-B, Category Fluency 'Low' performance on each defined by 'pre-validated cut-points': MMSE≤24, TMT-B≥300 seconds; Category Fluency ≤12
Van den Hurk et al. (2011)	Hoorn Study, Netherlands	Mean 6-year prospective	313	59-87 years (mean 67 years)	BNP, measured at baseline and follow-up	DSC, TMT-A, TMT-B, Stroop (parts I to III), Corsi-Block Tapping, RAVLT, Rey Complex Figure, Location Learning Test, verbal fluency, forward/ backward digit span. Administered at follow-up only.
Kindermann et al. (2012)	Consecutively enrolled patients with decompensated CHF, controls with stable CHF and CHF-free controls, Germany	Mean 14-day prospective on treatment effects (pre-test – treatment – post-test)	60	Mean 61 years	NT-proBNP	Digit Span, Logical Memory, Stroop, DSC, Raven's Standard Progressive Matrices Test
Nilsson et al. (2012)	Consecutively enrolled psychogeriatric patients (47% have dementia), Sweden	Cross-sectional	447	Median 75 years	NT-proBNP; 'vascular disease' defined as MI, angina, TIA, stroke, VaD, PAD, hypertension, atrial	1. MMSE; 2. Diagnosis with dementia or MCI 3. CT scan

					fibrillation	
Hu et al. (2012)	3 cohorts (267 from University of Pennsylvania 'Penn'; 333 from Washington University 'WU'; 566 from Alzheimer's Disease Neuroimaging Initiative (ADNI), North America	Cross-sectional	1166	≥50 years (means 65 to 72 years for cognitive groups)	Penn and WU: NT-proBNP; ADNI: BNP	3. Diagnosis with MCI, AD or other dementias; cognitively healthy controls 4. Measurement of beta amyloid and tau in CSF in ADNI cohort only
Soares et al. (2012)	Alzheimer's Disease Neuroimaging Initiative (ADNI) (396 patients with MCI, 112 patients with AD and 58 controls), North America	Up to 4-year prospective	566	≥60 years (mean 75 years)	NT-proBNP measured at baseline and year 1	Diagnosis with MCI, AD or cognitively unimpaired
Vecchio et al. (2012)	Older individuals (10 patients with CHF, 20 with AD and 20 controls), Italy	Cross-sectional	50	Mean 76 years	BNP	EEG recordings in resting state (authors note that high power of delta rhythms have been associated with cognitive impairment in the literature)
Hiltunen et al. (2013)	Kuopio 75+ (137 with cognitive disorder, 464 controls), Finland	5-year prospective	601	75-96 years (means 80 to 84 for cognitive groups)	BNP	Neuropsychological assessment at baseline and follow-up to diagnose normal cognitive function, AD, VaD, dementia with Lewy bodies, dementia due to medical condition

CVD, cardiovascular disease; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; DRS, Dementia Rating Scale; HDS-R, Revised Hasegawa Dementia Scale; CHF, congestive heart failure; VaD, vascular dementia; AD, Alzheimer's Disease; CT, computed tomography; DSC, Digit Symbol Coding; TMT-B, Trail-Making Test-B; RAVLT, Rey Auditory Verbal Learning Test; NT-proBNP, N-terminal pro-brain natriuretic peptide; BNP, brain natriuretic peptide; EEG, electroencephalograhy; CSF, cerebrospinal fluid.

Table 3.6: Main findings from studies identified in systematic search

	Finding	Limitations
Gunstad et al. (2006)	Inverse association between BNP and DRS (r=-0.34, p=0.02); BNP explained 9% of unique variance in DRS (p=0.017; adjusted for age, sex, demographic, 'clinical confounders')	
Mauro et al. (2007)	1. Higher mean BNP in MMSE≤24 versus MMSE>24 (Cohen's *d*=-0.44, p=0.05) 2. (Apparently) inverse association between BNP and MMSE (r=0.25, p=0.02) 3. Trend for inverse association between BNP and Corsi Block Tapping (r=-0.20, p=0.07)	Reported positive correlation between MMSE and BNP, but interpreted as inverse association
Nilsson et al. (2008)	1. Median NT-proBNP higher in patients with any pathological finding on CT compared with remaining sample (p<0.001) 2. No difference in NT-proBNP between group with cerebral infarction +/- white matter lesions and group with white matter lesions only; p>0.05) 3. 74% of VaD patients had 'elevated NT-proBNP', compared with 51% of AD patients and 41% of patients with depression	No statistical tests applied to group differences in 'frequency of elevated NT-proBNP'; 'elevated' not defined
Suwa & Ito (2008)	1. Mean BNP higher in group with 'cognitive impairment' than in remaining sample (Cohen's *d*=-0.72, p<0.007) 2. BNP >40 pg/ml associated with OR 9.74 for 'cognitive impairment' (p=0.011 in unadjusted analyses)	Used only MMSE
Daniels et al. (2009)	1. High NT-proBNP group (≥450pg/ml) was at risk of low MMSE (OR 2.04, p=0.01), low TMT-B (OR 1.83, p<0.01) and low Category Fluency (OR 1.54, p=0.048) when age and education were controlled for. Findings persisted for MMSE and TMT-B when vascular risk factors were additionally controlled for. 2. Findings unchanged when patients with stroke were excluded	Abstract only; did not specify 'low' cognitive scores (although these were subsequently specified in Daniels et al., 2011)
Kondziella et al. (2009)	Higher mean BNP in VaD group (but not AD group) compared with controls (Cohen's *d*=0.62, p=0.03)	
Naito et al. (2009)	Inverse association between BNP and HDS-R (r=-0.33, p=0.03)	
Vaes et al. (2009)	1. 27% (n=72) had MMSE<19 2. 18% (n=45) had diabetes 3. No association between sex-specific tertiles NT-proBNP and poor cognitive function (p=0.70)	

	4. No association between sex-specific tertiles NT-proBNP and diabetes (p=0.88)	
Kerola et al. (2010)	1. Cross-sectional analysis: Inverse association between BNP and MMSE (beta coefficient =-0.15, p=0.001) 2. Prospective analyses: Association between higher baseline BNP and steeper 5-year decline on MMSE (beta coefficient =0.14, p=0.019). Association between higher baseline BNP and increased risk of AD and VaD (for each SD increase in BNP OR 1.53 for any type of dementia, p=0.016 in model controlling for age, education, hypertension) (dementia total n=59) 3. Results similar following exclusion of CHF patients or exclusion of subjects with a history of stroke 4. No association between BNP and presence of diabetes	
Nilsson et al (2010)	1. Inverse association between NT-proBNP and MMSE (r=-0.1, p<0.001) 2. Lower MMSE in 'high' NT-proBNP group (>300 ng/l) than in 'low' group (median MMSE scores 22/30 versus 26/30, p<0.001) 3. Groups with WML, cerebral infarction or both on CT each had higher mean NT-proBNP compared with a group without pathological findings (all p<0.001)	
McDonagh et al. (2010)	No association between pre-surgery BNP and risk of POCD at either 6 weeks or 1 year after surgery (data not shown)	
Daniels et al. (2011)	1. High NT-proBNP group (≥450pg/ml, n=198) group had increased risk of 'low' MMSE (OR 2.04, 95% CI 1.18, 3.52), TMT-B (OR 1.84, 95% CI 1.26, 2.67); Category Fluency (OR 1.54, 95% CI 1.00, 2.36) in analyses controlling for age, sex, education. 2. Adjustment for vascular risk factors, coronary heart disease and stroke, exclusion of patients with coronary heart disease and/or stroke or exclusion of patients with stroke did not change results for MMSE and TMT-B, but rendered finding for Category Fluency non-significant (p>0.05) 3. High NT-proBNP group had lower scores on MMSE (Cohen's *d*=0.48, p<0.001), TMT-B (Cohen's *d*=-0.36, p<0.001), Category Fluency (Cohen's *d*=0.17, p=0.031) in analyses adjusted for age, sex, education compared with low NT-proBNP group (all except Category Fluency, p=0.08, survived adjustment for vascular risk factors, coronary heart disease and stroke	

Van den Hurk et al. (2011)	1. Baseline BNP predicted 3.6% of variance in processing speed, 3.3% in memory and 12.4% in attention/executive function. Follow-up BNP predicted 2.8% and 6.5% in speed and attention/executive function respectively (all p<0.05; all adjusted for age, sex, estimated pre-morbid ability, pre-diabetes, diabetes) 2. Association between an increase in BNP during follow-up and lower attention and executive function at follow-up (decline in BNP determined by adjustment of follow-up BNP for baseline BNP) (p<0.05)	
Kindermann et al. (2012)	1. Inverse association between NT-proBNP and Logical Memory, Digit Span, DSC, Stroop (range p<0.0001 to p<0.05) 2. Treatment ('compensation') improved cognitive function pre-test to post-test for the decompensated CHF group	Did not report effect sizes
Nilsson et al. (2012)	1. Group with vascular disease and elevated NT-proBNP had lower MMSE and higher prevalence of pathological findings on CT compared with group free of vascular disease and with normal NT-proBNP (both p<0.001) 2. Group free of vascular disease but with elevated NT-proBNP had similar MMSE and prevalence of pathological findings on CT as group free of vascular disease with normal NT-proBNP (both p>0.05) 3. Within MCI group (n=119), patients with vascular disease and elevated NT-proBNP had lower MMSE and higher likelihood of pathological findings on CT compared with patients free of overt vascular disease and normal NT-proBNP (both p<0.01) 4. No associations between NT-proBNP groups and MMSE or pathological findings in VaD group (n=89) (all p>0.05) 5. In AD group (n=77), association between elevated NT-proBNP (irrespective of overt vascular disease) and higher likelihood of pathological findings on CT (p<0.05), but no association with MMSE (p>0.05)	
Hu et al. (2012)	1. Penn: No association of NT-proBNP with very mild dementia/MCI/AD (p=0.08) (adjusted for age, sex) 2. WU: Association of NT-proBNP and very mild dementia/MCI/AD (OR 1.07, p<0.001) (adjusted for age, sex) 3. ADNI: Association of BNP with very mild dementia/MCI/AD (OR 1.23, p<0.001) (adjusted for age, sex)	

	4.ADNI: Association of BNP with Aβ42 (<193 pg/ml) (OR 1.75, $p<0.001$) and t-tau/Aβ42 ratio (>39) (OR 1.11, $p<0.001$) (adjusted for age, sex)	
Soares et al. (2012)	1. Cross-sectional at baseline: compared with controls, higher mean NT-proBNP in MCI ($p<0.001$) and AD ($p<0.001$) 2. Cross-sectional at year 1: compared with controls, higher mean NT-proBNP in MCI ($p=0.001$) and AD ($p=0.003$) 3. In analysis of a screening tool to differentiate AD from healthy individuals, inclusion of plasma markers (including NT-proBNP) improved specificity from 40% (on basis of age, sex, APOE e4 status) up to 80%	1. Did not report prospective associations or effect sizes 2. Reported data from same cohort as Hu et al. (2012), but measured NT-proBNP instead of BNP
Vecchio et al. (2012)	In CHF patients: Association between BNP and amplitude of global delta source on EEG ($r=0.77$, $p=0.009$) (unadjusted)	Administered cognitive tests but did not report associations with BNP
Hiltunen et al. (2013)	1. Cross-sectional: risk of any dementia associated with BNP was increased in youngest tertile (OR 1.44, 95% CI 1.01, 2.07, $p=0.047$), non-significant in middle tertile ($p=0.49$) and reduced in oldest tertile (OR 0.57, 95% CI, 0.32, 1.00, $p=0.050$) (adjusted for age, sex, medication, heart failure, blood pressure; findings similar when excluding subjects who die within 12 months of baseline) 2. Prospective: in previously unimpaired subjects, baseline BNP predicted risk of dementia during follow-up in youngest tertile only (OR 1.89, 95% CI 1.07, 3.34, $p=0.028$) (adjusted for age, education, hypertension)	1. Unclear reason for varying choice of covariates 2. Reported data from same cohort as Kerola et al. (2010)

DRS, Dementia Rating Scale; MMSE, Mini Mental State Examination; DSC, Digit Symbol Coding; CHF, congestive heart failure; WML, white matter lesions; CT, computed tomography; POCD, post-operative cognitive dysfunction; VaD, vascular dementia; AD, Alzheimer's Disease; SD, standard deviation; MCI, mild cognitive impairment; NT-proBNP, N-terminal pro-brain natriuretic peptide; BNP, brain natriuretic peptide; OR, odds ratio; CSF, cerebrospinal fluid; APOE e4, apolipoprotein e4 allele.

Overview of included studies

The 19 studies reported results from 14 cohorts, and reported data for a total of 7438 participants. One study was in abstract form and was later followed by a full report; the remaining articles were full studies. All samples included both males and females. Seven studies assessed middle-aged to older cohorts form the general population, 6 recruited either patients with diagnosed dementia or recruited subjects from psychogeriatric facilities, 5 studies included individuals with cardiovascular disease and 1 was of patients undergoing non-cardiac surgery. All except 5 (with 6-

month to 6-year follow-up) had cross-sectional designs. One additional prospective study on treatment effects had a 14-day pre-test to post-test period, but reports data on natriuretic peptides and cognitive test performance at pre-test phase only, i.e. cross-sectionally. Mean age of participants (where reported) ranged between 61 years and 84 years. Nine studies investigated NT-proBNP; 9 studies assessed its correlate BNP. One study reported data from 3 cohorts of which 2 measured NT-proBNP and 1 measured BNP. Seven studies were on associations of natriuretic peptides with diagnosis of dementia (either with or without additional cognitive assessment on screening instruments). One of these 7 studies also measured beta amyloid and tau in participants' cerebrospinal fluid. Eleven studies employed detailed neuropsychological tests, screening instruments for dementia and dementia rating scales, with brain imaging data (computed tomography, CT) additionally available in 3 studies. Finally, 1 study related BNP to findings on EEG, but not to cognitive function. Due to the apparent heterogeneity between the studies identified in the search in terms of study design and cognitive outcome, meta-analysis to derive overall effect sizes of associations was not possible. Findings are therefore described in narrative form.

Findings from included studies

A majority of the studies identified in the search reported significant findings for associations between higher natriuretic peptides and worse cognitive outcome. In a Swedish cohort of dementia-free older adults and dementia patients, as well as in a study of patients with heart failure, NT-proBNP or BNP correlated inversely with scores on the MMSE, each with small to medium effect sizes (Mauro et al., 2007; Nilsson, Gustafson, & Hultberg, 2010). Each unit increase in BNP was linked to a loss of 0.15 points on the MMSE in an analysis of the Finnish Kuopio 75+ cohort of relatively old dementia-free adults (Kerola et al., 2010). In a smaller study of heart failure patients and healthy individuals, NT-proBNP levels further correlated negatively with performance on a range of more detailed cognitive tests, although effect sizes were not reported and could not be derived on the basis of reported statistics (Kindermann et al., 2012). Significant differences in mean natriuretic peptide levels between higher cognitively functioning and lower functioning groups have also been reported in relatively small studies of patients with heart failure

(Mauro, et al., 2007) or cardiovascular disease (Suwa & Ito, 2009), with unadjusted medium to large effect sizes. Conversely, in a study of psychogeriatric patients, a group with elevated NT-proBNP also had lower cognitive function when compared with patients with NT-proBNP below a pre-specified cut-point (Nilsson, et al., 2010). Finally, BNP correlated strongly with the delta source amplitude on EEG, which had previously been linked to abnormal cerebral blood flow and reduced cognitive function (Vecchio et al., 2012).

Age, which may affect both natriuretic peptide levels and cognitive function, was not controlled for in any of these investigations, but similar results have been reported in studies which apply adjustment. In one relatively small study of dementia-free cardiovascular disease patients, BNP correlated inversely with scores on a brief cognitive screening test with a medium effect size and accounted for as much as 9% of variance even after multivariable adjustment (Gunstad et al., 2006). Following adjustment for age, sex and education, a ‘high’ NT-proBNP group was at around two-fold increased risk of ‘low’ performance on the MMSE and on a trail-making test of executive function and visual attention when compared to a ‘low’ NT-proBNP group in the Rancho Bernardo Study. When cognitive test performance was treated as continuous, significant group differences of medium effect size were also reported, although findings for a verbal fluency test of executive function were less consistent (Daniels et al., 2009; Daniels et al., 2011). Because all significant results persisted when analyses were additionally adjusted for stroke and when cardiovascular disease patients were excluded (Daniels, et al., 2011), the study supports a role of NT-proBNP in late-life cognitive function which is over and above that of being a marker of symptomatic macrovascular disease.

In contrast, results from the Swedish cohort showed confounding of associations between the peptide and cognition by symptomatic macrovascular disease. Individuals with pathological findings on CT had higher mean NT-proBNP compared with the remaining population (Nilsson, Gustafson, & Hultberg, 2008; Nilsson, et al., 2010), but a group with elevated NT-proBNP who was free of symptomatic macrovascular disease had similar MMSE scores and prevalence of pathological findings on CT as a group with normal NT-proBNP who was also free of symptomatic disease. The only significant association between NT-proBNP and pathological findings which were independent of symptomatic macrovascular disease was reported for a group with Alzheimer's Disease, and these findings did not extend to associations with cognitive test scores (K. Nilsson, L. Gustafson, & B. Hultberg, 2012). Further speaking against associations with cognitive outcome, one relatively large study failed to establish cross-sectional associations between tertiles of NT-proBNP and performance on the MMSE (Vaes, de Ruijter, Degryse, Westendorp, & Gussekloo, 2009).

Yet, further cross-sectional analyses have associated natriuretic peptides with presence of dementia. In unadjusted analyses of one case-control study, individuals with diagnosed vascular-type dementia (but not those with Alzheimer's Disease) had higher mean BNP levels compared with dementia-free individuals, with a medium effect size (Kondziella, et al., 2009). 'Elevated' serum NT-proBNP was prevalent in as many as 74% of patients with vascular dementia in the Swedish cohort, but statistical testing was not applied to a comparison with controls (Nilsson, et al., 2008). In two US cohorts, BNP was found to be higher in patients with diagnosis of dementia or milder forms of impairment compared with controls (W. T. Hu et al., 2012). In the North American Alzheimer's Disease Neuroimaging Initiative (ADNI), subjects with dementia or milder forms of impairment both also had higher NT-proBNP (Soares et al., 2012) and BNP (W. T. Hu, et al., 2012) compared with controls. Additionally, BNP levels were associated with presence of Alzheimer's Disease-typical protein in cerebrospinal fluid (W. T. Hu, et al., 2012). Addition of a range of biomarkers (including NT-proBNP) to a statistical model attempting to identify patients with Alzheimer's Disease versus dementia-free indiviudals further increased specificity, although the role specifically of NT-proBNP in this finding is unclear (Soares, et al., 2012). In a Japanese sample of patients with diagnosed dementia, BNP also correlated inversely with scores on a cognitive test aimed at determining the severity of dementia, with a medium effect size in the association (Naito, Naka, & Watanabe, 2009) A more complex picture of associations of natriuretic peptides with dementia emerged in the Kuopio 75+ cohort. Here, higher

BNP was linked to presence of dementia in the youngest tertile, but was associated with a reduced likelihood of dementia in the oldest tertile aged 83 to 96 years (Hiltunen, et al., 2013). The authors concluded that the development of cognitive impairment may decrease the levels of BNP, thereby implying causality, but did not back up this claim by specifying potential mechanisms.

None of the studies identified in the search assessed peak cognitive ability in young adulthood as a potential determinant of late-life natriuretic peptides. If such associations exist (which is likely given that pre-morbid ability predicts risk of late-life symptomatic macrovascular disease), these could account for any cross-sectional links between natriuretic peptides and cognition. Prospective evidence is currently limited to four investigations reporting data from three cohorts. In one study of dementia-free older adults, baseline BNP accounted for around 4% of variance in processing speed, for 3% of memory and 12% of attention and executive functioning at six-year follow-up when a number of covariates including diabetes and an estimate of peak pre-morbid ability were controlled for. This suggests a steeper estimated lifetime decline in cognitive function in individuals with higher late-life BNP. An increase in BNP between baseline and follow-up additionally predicted a lower level of executive function and attention at follow-up (van den Hurk et al., 2011). With respect to assessments of prospectively ascertained change in cognitive function, the literature has produced inconsistent findings. Higher baseline BNP predicted steeper subsequent decline on the MMSE over five years in Kuopio 75+. Additionally, each standard deviation increase in baseline BNP increased the risk of dementia during follow-up by 55% in initially non-demented individuals (Kerola, et al., 2010), although a re-analysis which stratified by age group found that the predictive ability of BNP was restricted to the youngest tertile of this relatively old cohort (Hiltunen, et al., 2013). A prospective investigation of around 400 older US adults, who were cognitively tested prior to, six weeks after and one year after non-cardiac surgery, baseline BNP was unrelated to the risk of ‘postoperative cognitive dysfunction’ defined as a decrease in at least one standard deviation compared to pre-operative assessment in at least one cognitive domain at either of the two follow-up assessments (McDonagh et al., 2010).

Conclusions

In contrast to the remaining sections of this chapter, which focused on studies of older adults with diabetes, the systematic review of natriuretic peptides included studies of the general older and other patient populations, because although one study identified in the search controlled for presence of diabetes in their analyses of BNP and cognitive function, investigations of cohorts consisting of people with diabetes were previously neglected. In the studies identified in the search, the balance of evidence overall suggests a potential association between natriuretic peptide levels and poorer cognitive outcome both in terms of impairment short of dementia and frank dementia. Yet, the role of confounding in many of these studies, including by individual differences in peak pre-morbid ability, is unclear, and investigations with prospective designs have produced conflicting results.

Potentially asymptomatic PAD and carotid intima-media thickness (cIMT)

Potentially asymptomatic PAD and diabetes

A low ABI calculated from systolic pressure in the ankle and brachial pressure indicates potentially asymptomatic PAD as well as generalised atherosclerosis (C. Hayashi et al., 2004). The measure is non-invasive, and has been found to be 95% sensitive and almost 100% specific in the identification of patients with PAD. The classification of PAD according to ABI is as follows (ADA, 2003):

- Normal: 0.91 – 1.30
- Mild obstruction: 0.70 – 0.90
- Moderate obstruction: 0.40 – 0.69
- Severe obstruction: <0.40

Values >1.30 are common in diabetes, but indicate poorly compressed arteries in the lower limbs and are therefore unreliable measurements of ABI in this type of patient (ADA, 2003; Potier, et al., 2011). Low ABI and progressive worsening of ABI both predict poor health outcomes (Banerjee, et al., 2010). Individuals with ABI ≤0.90, which was present in 9% of older individuals in one Scottish cohort (Fowkes et al., 1991), are at two-fold risk of cerebral infarction (Banerjee, et al., 2010) and ten-year mortality (Ankle Brachial Index Collaboration, 2008). The less frequently used lower limb arterial duplex or the cardio-ankle vascular index (CAVI), which is calculated from the ankle and brachial pressures, a microphone for detecting heart beats and electrocardiograph electrodes placed on wrists, may be used as alternatives to the ABI to ascertain potentially asymptomatic arterial disease in the periphery. In contrast to ABI, higher CAVI values indicate higher severity of disease.

Consistent with the aforementioned increased prevalence of symptomatic PAD in patients with type 2 diabetes, ABI is abnormal in around 15% of diabetes patients (Mukherjee, 2009), and some evidence suggests that CAVI may be higher in patients with diabetes than in non-diabetic individuals (H. Wang et al., 2013).

Carotid artery intima-media thickness and diabetes

Carotid arteries consist of three layers (innermost intima, media and outermost adventitia) and run along the axis of the neck at a depth of 2 to 4 cm (Molinari, Zeng, & Suri, 2010). The distance between the lumen-intima interface and the media-adventitia interface defines cIMT; thickness >0.9 mm is considered indicative of atherosclerosis (Haley, Tarumi, Gonzales, Sugawara, & Tanaka, 2010). Carotid IMT can be visualised non-invasively in 2-dimensional images using ultrasound and predicts cardiovascular outcomes. One meta-analysis reports 15% increased risk for MI and 18% increased risk for stroke per 0.1 mm increase (Lorenz, Markus, Bots, Rosyall, & Sitzer, 2007). As well as increasing with age (Ludwig, von Petzinger-Kruthoff, von Buguoy, & Stumpe, 2003), cIMT is raised in (pre-)diabetes. A systematic review found an average of 0.13 mm and 0.04 mm thicker cIMT in populations with diabetes and IGT respectively compared with normoglycaemic controls (Brohall, Odén, & Fagerberg, 2005).

Potentially asymptomatic PAD, cIMT and cognition in type 2 diabetes

A systematic search of the literature on cross-sectional or prospective studies with observational designs investigating potentially asymptomatic atherosclerosis determined by cIMT or by ABI, CAVI or lower limb arterial duplex and cognition in type 2 diabetes was performed. Where the evidence from studies of people with diabetes is lacking, the literature on the general population was consulted. However, potential differences between people with diabetes and the general population in terms of atherosclerosis associations with cognition are not at the centre of the research question and are therefore not fully addressed.

Search strategy

The titles, abstracts and keywords (.mp) of studies indexed by Medline and published between 1946 and the first week of May 2013 were searched. The search terms were "type 2 diabetes" OR "non-insulin dependent" OR NIDDM, as well as "intelligence" OR "cognitive function" OR "cognitive abilit*", and "carotid intima-media" OR "cIMT" OR "ankle-brachial pressure index" OR "ankle-brachial index" OR "ABI" OR "arterial duplex" OR "cardio-ankle vascular index". The three separate searches were combined using AND. Reference lists of reviews of the literature on non-diabetic populations and key papers were scanned, and experts in the field were consulted.

Selection of studies

Inclusion criteria were: a) original studies, b) cohort studies either exclusively of humans with type 2 diabetes, or unspecified diabetes and mean age >55 years, or studies reporting data for a diabetic subsample, c) measured cIMT and/or peripheral arterial disease using ABI, CAVI or lower limb arterial duplex, d) application of neuropsychological tests or clinical evaluation of dementia and f) reporting of data linking cIMT and/or peripheral arterial disease with cognitive function. Case studies and reviews of the literature were excluded from the review.

Data extraction

Titles and abstracts of all studies identified by the search were screened. Full texts were accessed for those which appeared to meet the inclusion criteria or where this could not be determined on the basis of the abstract. Data from studies which met the criteria were extracted. Information on study design, total number of participants, age at baseline (range or mean), the assessment of cIMT/ABI/CAVI/lower limb arterial duplex, the measurement of cognitive function, main findings including effect sizes and p-values or confidence intervals where available, and potential limitations was tabulated.

Results

The search resulted in 10 studies and the full texts of 9 articles were accessed (Figure 3.4). Of these, one described a study protocol and two studies reported data on cIMT and cognition, but failed to explore the data for associations and so were excluded. Consequently, a total of 6 articles were included in this review. The extracted data is presented in Table 3.7 and Table 3.8.

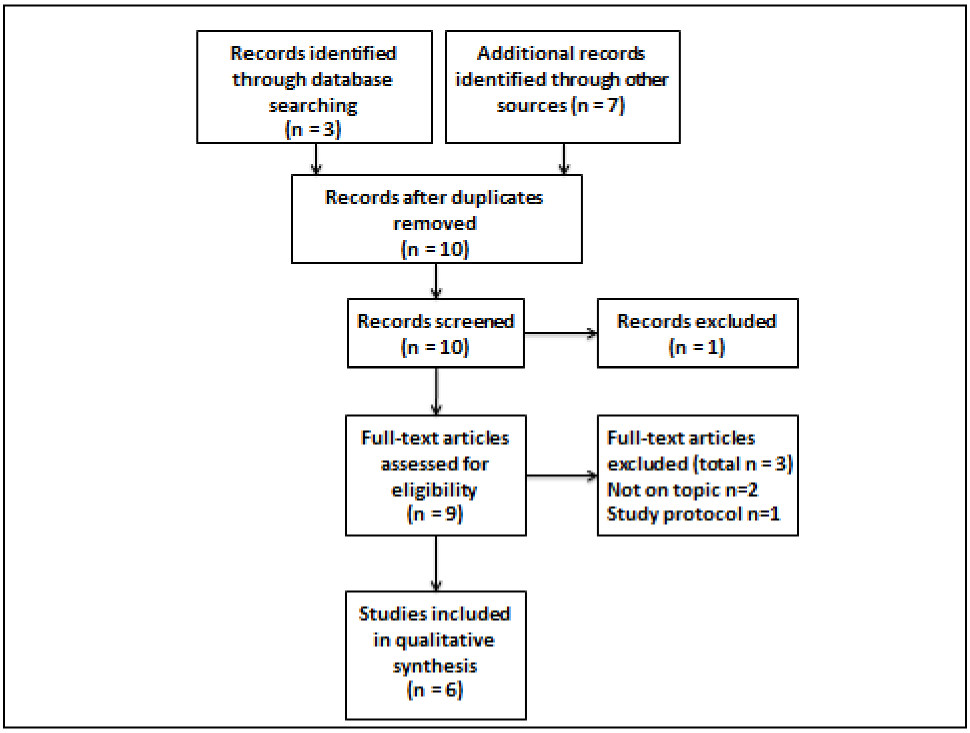

Figure 3.4: Systematic literature search on cIMT or potentially asymptomatic PAD and cognition in type 2 diabetes

<u>Table 3.7: Summary of studies identified in systematic search on cIMT/ potentially asymptomatic PAD and cognition in type 2 diabetes or older adults with any type of diabetes</u>

	Sample	Design	Total n	Age at baseline	cIMT/ potentially asymptomatic PAD	Measurement of cognitive function
Manschot et al. (2007)	Utrecht Diabetic Encephalopathy Study (112 patients with type 2 diabetes, 56 controls; all free of dementia or disease affecting cognitive function), Netherlands	Cross-sectional	168	55-80 years (mean 66 years)	cIMT	Composite z score calculated from 11 cognitive tests of domains memory, reasoning, processing speed, attention/executive function, visuospatial construction. Dutch version of NART
Bruce et al. (2008) Diabetologia	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Median 8-year prospective	302	≥70 years (mean 76 years)	'PAD' defined as ABI ≤0.90 or lower-limb amputations due to diabetes	1. MMSE, CDR, IQCODE 2. neuropsychological assessment. MCI defined as CDR=0.5 without dementia; 'cognitive decline' defined as conversion between normal cognition, MCI and dementia
Bruce et al. (2008) Diabetes Care	Fremantle Diabetes Study (patients with any type of diabetes), Australia	Median 2-year prospective	205	≥70 years (mean 75 years)	'PAD' defined as ABI ≤0.90 or lower-limb amputations due to diabetes	1. MMSE, CDR, IQCODE 2. neuropsychological assessment.

						MCI defined as CDR=0.5 without dementia; ‘cognitive decline’ defined as conversion between normal cognition, MCI and dementia
Chen, G. et al. (2011)	Hospitalised patients with type 2 diabetes (free of central nervous system disease, head trauma, inflammatory disease, infectious brain disease, drug/alcohol abuse), China	Cross-sectional	101	Mean 63 years	cIMT, ABI	MoCA; ‘MCI’ defined as MoCA<26
Amer et al. (2012)	Patients with diabetes (any type) and PAD versus diabetes only versus controls (all MMSE>24), Egypt	Cross-sectional	90	≥60 years	‘PAD’ defined as presence of mild/moderate/severe stenosis on lower limb arterial duplex	MMSE; self-reported education
Chen, R.-H. et al. (2012)	Patients with type 2 diabetes, China	Cross-sectional	157	40-69 years (mean 55 years)	cIMT (average and maximum)	‘MCI’ defined as MoCA<26 with CDR ≥0.5 (n=93); ‘normal’ defined as MoCA≥26 (n=64)

MoCA, Montreal Cognitive Assessment, MCI, mild cognitive impairment; cIMT, carotid intima-media thickness; ABI, ankle brachial pressure index; PAD, peripheral arterial disease; NART, National Adult Reading Test.

Table 3.8: Main findings from studies identified in systematic search

	Finding	Limitations
Manschot et al. (2007)	Diabetic group: cIMT entered in regression model explaining cognitive test performance (adjusted for age, sex, NART), but apparently not retained in model	Data not shown
Bruce et al. (2008) Diabetologia	1. Cross-sectional analysis: prevalence of PAD in cognitively normal (38%) < MCI patients (45%) < dementia patients (75%) (p=0.001 for trend) 2. Cross-sectional analysis: PAD associated with OR 2.03 for all MCI/dementia and OR 4.54 for all dementia (both p<0.05; PAD unrelated to MCI or AD alone) 3. Prospective analysis: prevalence of baseline PAD in cognitively normal (21%) < MCI patients (27%) < dementia patients (57%) at follow-up (p=0.001 for trend) 4. Prospective analysis: baseline PAD associated with OR 2.23 for any MCI/dementia (p<0.01) and 5.35 for all dementia at follow-up (p<0.001) (PAD unrelated to risk of MCI or AD alone). All analyses unadjusted.	Did not explore change in cognitive function between baseline and year 8; did not adjust for potential confounders
Bruce et al. (2008) Diabetes Care	1. Prospective analysis of whole sample: prevalence of baseline PAD similar in patients who 'declined' and others (44% versus 36%; p=0.43) 2. Similar results when analyses of cognitive decline restricted to individuals with 'normal cognition' at baseline	Short follow-up period
Chen, G. et al. (2011)	1. Lower ABI in MCI group compared with others (mean 1.09 versus 1.17; Cohen's *d*=-0.78, p<0.01) 2. Higher cIMT in MCI group compared with others (mean 1.19 mm versus 1.05 mm; Cohen's *d*=0.78, p<0.01	1. Associations of ABI/ cIMT with age not reported; analyses not controlled for age (but age not significantly associated with MCI) 2. High prevalence of MCI (n=58; 57%) considering young age
Amer et al. (2012)	1. Illiteracy in 67% of diabetics with PAD, in 63% of diabetics without PAD and 27% of non-diabetic controls (p<0.001 across groups)	1. Did not report associations between PAD and MMSE 2. Did not apply pairwise group comparisons
Chen, R.-H. et al. (2012)	Group with MCI had higher average cIMT and max cIMT compared with remaining population (max cIMT 0.91 mm versus 0.81 mm; Cohen's *d*=0.67, p<0.001) Inverse correlation between MoCA scores and max cIMT (r=--0.25, p=0.005)	High prevalence of MCI (n=93; 59%) considering young age

MoCA, Montreal Cognitive Assessment; MCI, mild cognitive impairment; cIMT, carotid intima-media thickness; ABI, ankle brachial pressure index; AD, Alzheimer's Disease; PAD, peripheral arterial disease.

Overview of included studies

The 6 studies included in the review reported findings from 5 cohorts. The total number of participants included in these studies was 1023 and all included both males and females. Mean age of participants (where reported) ranged between 55 years and 75 years. Three of the studies included only patients with diagnosis of type 2 diabetes. The remaining 3 studies included older patients with diabetes of any type, but given the advanced age of the samples may be assumed to have captured mainly people with type 2 diabetes. Three studies focused on PAD defined either by ABI or lower limb arterial duplex, two studies assessed cIMT and one study included data on both ABI and cIMT. CAVI was not measured in any of the studies. Three of the 6 studies were cross-sectional and 2 (both of which reported data from the same cohort) were prospective with median follow-ups of 2 and 8 years respectively, although only the 2-year prospective study investigated cognitive change during the follow-up period. The 8-year prospective study associated baseline predictors with cognitive status at follow-up, and so essentially reports cross-sectional analyses. These 2 studies were also the only ones which included both cognitive screening instruments and clinical diagnosis of dementia. Of the remaining 4 studies, 3 were based on brief cognitive screening instruments and 1 applied more detailed neuropsychological tests. Due to the small number of studies identified in the systematic search and heterogeneity in terms of study design and cognitive outcomes, meta-analysis to derive average effect sizes of associations was deemed not possible and so findings from the included studies are described in narrative form.

Findings from included studies

Potentially asymptomatic PAD and cognition

In the Fremantle Diabetes Study, which provided data for 2 of the 5 studies identified in the search 38% of cognitively 'normal' individuals, 45% of individuals with reduced cognitive function short of dementia and 75% of dementia patients suffered from potentially asymptomatic PAD (ABI ≤0.90 or diabetes-related lower-limb amputation). Across groups, the difference in prevalence reached statistical significance, although the authors did not apply pairwise comparisons. Overall, presence of potentially asymptomatic PAD was associated with an unadjusted two-fold likelihood of presence of any cognitive impairment and almost five-fold likelihood of presence of dementia. Results were similar in further cross-sectional analyses of baseline PAD and cognitive function assessed after a median of eight years. Baseline PAD was prevalent in 21% of individuals with 'normal' cognition at eight-year follow-up, in 27% of people with reduced cognitive function short of dementia and in 57% of individuals with dementia diagnosis by the time of the eight-year follow-up; the risk of any cognitive impairment was again around two-fold and the risk of dementia was more than five-fold (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008). The findings were supported by a Chinese study of hospitalised type 2 diabetes patients. Mean ABI was lower in a group with reduced cognitive function compared with a group with 'normal' cognitive function as defined on the basis of a screening instrument, with a large effect size in the unadjusted group difference in means (G. Chen, et al., 2011).

Such reports may be driven by increased risk of both PAD and poorer late-life cognitive outcome in diabetes patients with lower peak ability in young adulthood compared with higher-ability patients. Findings from an Egyptian case-control study of older people with diabetes and non-diabetics support this possibility. Although the authors failed to associate cognitive function in the sample with evidence of PAD on a lower limb arterial duplex, links between PAD, diabetes and education were explored. Whereas 67% of diabetics with PAD were illiterate, this applied to 63% of diabetics without PAD and 27% of controls (Amer, Alsadany, Tolba, & Omar, 2013). Pairwise comparisons were not carried out, but at least a trend for detrimental effects of illiteracy on risk of PAD becomes apparent in the diabetes patients of the study.

In support of confounding of cross-sectional associations between PAD and cognition, the only truly prospective investigation of PAD and cognitive decline reported non-significant associations. During two years of follow-up in the Fremantle Diabetes Study, potentially asymptomatic PAD at baseline (ABI ≤0.90 or diabetes-related lower-limb amputation) was unrelated to risk of conversion between 'normal' cognition, reduced cognitive function short of dementia and diagnosis with dementia. Findings were similar when analyses were restricted to individuals with initially 'normal' cognition at baseline, although the very short follow-up period clearly restricts the interpretations made on the basis of this finding (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008).

Carotid IMT and cognition

In two of the studies on cIMT and cognition identified in the search, participants were grouped according to performance on a brief cognitive screening instrument, the Montreal Cognitive Assessment (MoCA). In the analyses, which were both set in China and which appeared to differ mainly in terms of the mean age of the sample, the respective group with lower cognitive function had higher mean cIMT compared with the remaining participants, with medium effect size in the sample with mean age 55 years (R. H. Chen, et al., 2012) and medium to large effect size in sample with mean age 63 (G. Chen, et al., 2011). Additionally, MoCA scores also correlated negatively with cIMT with a small to medium effect size in the study with a mean age of 55 years (R. H. Chen, et al., 2012). Neither of the studies applied adjustment for potential confounding variables. The only study on cIMT and cognitive decline identified in the search was a cross-sectional analysis adjusting for an estimate of peak pre-morbid ability. Here, cIMT appeared to be unrelated to estimated lifetime cognitive decline, although the finding is severely limited in that no data or findings from unadjusted analyses were reported (Manschot, et al., 2007).

Comparison of findings from systematic search with evidence in the general population

With the restriction of the evidence to five studies, the findings from the systematic search were extended through the consultation of the literature on the general population.

Potentially asymptomatic PAD and cognition in the general population

As was found in the cross-sectional studies of diabetes patients identified in the search, a low ABI has also been linked to lower late-life cognitive function short of dementia in the general population (W. Johnson, Price, Rafnsson, Deary, & Fowkes, 2010; J. F. Price et al., 2006). In a Japanese study which categorised individuals according to cognitive function, ABI in the first versus the third tertile of the distribution was also linked to an odds ratio of 3.2 for presence of reduced cognitive function (MMSE<24), for instance. However, similar results were not found when analyses were unadjusted, or in comparison of the second to the third tertile of ABI (Sugawara et al., 2010). No significant associations between ABI<0.90 and performance on a battery of detailed neuropsychological tests were reported in a Dutch study of 400 middle-aged to older men (Muller, Grobbee, Aleman, Bots, & van der Schouw, 2007). Another study, which reports lower cognitive function in older adults with any PAD (ABI<0.97 after rest or <0.85 after treadmill test, or intermittent claudication identified using a questionnaire or on treadmill test) compared with controls who were free of PAD failed to stratify the analyses by PAD stage so that the role of potentially *asymptomatic* PAD as signified by the ABI is unclear in their results (Waldstein, et al., 2003).

Evidence from prospective associations of potentially asymptomatic PAD in general older populations also appears mixed. In an early analysis of the Cardiovascular Health Study of over 5000 older adults, an ABI<0.90 was associated with steeper decline on a test of processing speed and on the MMSE during seven years of follow-up (Haan, et al., 1999). Yet, mirroring the null findings of the only prospective investigation on the topic in people with diabetes (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008), baseline ABI was unrelated to change in cognitive function during the subsequent ten years in the Edinburgh Artery Study of middle-aged to older adults (W. Johnson, et al., 2010). This was in spite of previous evidence of steeper lifetime cognitive decline (estimated by adjustment for a test of vocabulary) in low ABI-individuals in this cohort (J. F. Price, et al., 2006). Despite equivalent baseline cognitive function in groups with lower or higher CAVI, scores on the MMSE and another dementia screening instrument for the 'low CAVI' group also declined at steeper rates relative to the 'high CAVI' group over four years in a Japanese study of over 75 year olds (Yamamoto, Yamanaka, Ishikawa, et al., 2009). In the Rotterdam Study, low ABI (<0.90) at baseline was unrelated to incidence of dementia during nine years of follow-up (van Oijen et al., 2007). Similar null findings were reported during eight-year follow-up in the Honolulu Asia Aging Study when ABI was treated as a continuous measure, but this finding may have been confounded by the aforementioned reduced validity of higher values of ABI. When individuals with ABI>1.20 were excluded from the analyses, ABI<0.90 predicted a 66% increased risk of any type of dementia and a 125% increased risk of vascular dementia during follow-up when age, education and vascular risk factors were controlled for (Laurin, Masaki, White, & Launer, 2007). A review on symptomatic and asymptomatic PAD and a systematic review of PAD defined by ABI ≤0.90 both support associations with reduced cognitive function and cognitive decline (Guerchet et al., 2011; Rafnsson, Deary, & Fowkes, 2009). In further support of these conclusions, the presence of PAD (independently of pre-morbid ability) has been linked to lower amplitude of the P300 component in middle aged to older adults (Kügler, Vlajic, Funk, Raithel, & Platt, 1995).

Carotid IMT and cognition in the general population

In the general population, cIMT-cognition associations appear well-established. In a French study, cIMT correlated negatively with level of cognitive function, although the finding was of small effect size and restricted to males with a presence of plaques in the carotid artery (Auperin et al., 1996). Another relatively small cross-sectional investigation reported associations of small to medium effect size with processing speed, executive functioning and attention, but non-significant associations with other cognitive domains (R. A. Cohen, et al., 2009). Directly contrasting this pattern of results, a Dutch study of 400 middle-aged to older males established cross-sectional associations with memory ability (each mm increase in cIMT was associated with 1.3-point lower memory score), but cIMT was unrelated to processing speed or executive function (Muller, et al., 2007). An association with the latter has since been supported (Semplicini et al., 2011). In a large study of younger to older individuals (21 to 84 years), higher cIMT was related to lower MMSE, lower processing speed, executive function and psychomotor control. For instance, each mm increase in cIMT was associated with 0.6 lower points on the MMSE (Zhong et al., 2011). One cross-sectional investigation also associated cIMT with memory ability and processing speed measured twelve years later (Komulainen et al., 2007). Further associations have been reported between higher cIMT and lower cognitive test performance in middle-aged to older patients with HIV (Becker et al., 2009), cardiovascular disease (Haley et al., 2007), and major depressive disorder (P. J. Smith et al., 2007). It is noteworthy that many of these studies controlled for vascular risk factors and for presence cardiovascular disease.

However, some studies have failed to establish such results (Auperin, et al., 1996; Romero et al., 2009; Yaldizli et al., 2006). Any cross-sectional associations may also be confounded by evidence of reduced peak pre-morbid ability or its proxies in individuals with higher cIMT (Knox et al., 2012; Singh-Manoux et al., 2008), but are supported by a number of prospective investigations. In the Baltimore Longitudinal Aging Study of middle-aged adults, baseline cIMT predicted (up to) eleven-year decline on some measures of verbal and non-verbal memory and executive function independent of covariates including education (indexing pre-morbid ability), although it was unrelated to trajectories in working memory or naming (Wendell, Zonderman, Metter, Najjar, & Waldstein, 2009). Two relatively small studies also reported links between baseline cIMT and rate of decline on screening instruments for dementia over one and two years in patients with Alzheimer's Disease and an unselected older population, respectively (Sander et al., 2010; Silvestrini et al., 2009).

Higher cIMT further predicted increased risk of two-year incidence of reduced cognitive function (defined using a screening instrument) in a German study of over 3000 older individuals (Sander, et al., 2010). In a smaller Greek study, each standard deviation increase in cIMT at baseline was associated with a two-fold risk of similarly defined reduced cognitive function one year after patients suffered their first-ever stroke (Talelli et al., 2004). Finally, subjects in the 5th quintile of baseline cIMT were at 50% increased risk of dementia during the subsequent nine years in the Rotterdam Study (van Oijen, et al., 2007). A review of the prospective literature in the general population concludes that higher cIMT is relatively consistently associated with lower cognitive function and with increased risk of dementia (Arntzen & Mathiesen, 2011), and a systematic review reports that 14 of 20 studies (of which twelve were cross-sectional and of which most employed the MMSE) reported significant associations of higher cIMT and lower cognitive function (Saleh, 2010). Yet, in the apparently only study of cIMT and cognition in people with type 1 diabetes, the DCCT/EDIC, cIMT was unrelated to 18-year decline in any of eight cognitive domains, despite a non-significant trend linking higher cIMT to steeper decline in psychomotor speed (Jacobson et al., 2011).

Conclusions

Overall, potentially asymptomatic PAD appears to be relatively consistently associated with poorer cognitive outcome including steeper cognitive decline in the general population. Although the only prospective analysis of the topic in patients with type 2 diabetes to date failed to establish significant associations, it is likely that asymptomatic PAD has a similarly useful role as a potential marker of risk in this type of patient.

Carotid IMT also appears to be a valuable marker predictive of late-life cognitive outcome in the general population. Despite a current lack of evidence, a similar role of cIMT as a marker for risk of cognitive decline in people with type 2 diabetes is plausible. Non-significant findings in the DCCT/EDIC trial of patients with type 1 diabetes may in part be accounted for by the relatively younger age of these patients.

3.3 Aims and Objectives

A number of potential risk factors for poorer late-life cognitive outcome have been identified in the general older population. Most studies treat presence of type 2 diabetes as a single risk factor in comparisons with non-diabetic controls or adjustment of analyses for diabetes status, rather than exploring associations between risk factors and cognitive outcome in exclusively diabetic populations. As the systematic review of the literature presented in this chapter has shown, this is particularly the case for investigations of macrovascular disease and cognition. The literature has also neglected the study of the relationship of hypoglycaemia with cognitive decline in people with type 2 diabetes. Finally, despite inter-relationships between the various factors, a majority of studies investigate individual risk factors in isolation in terms of their links with cognition and have failed to compare a wider range of risk factors in their predictive ability for cognitive decline.

3.3.1 Aims

The aim of the analyses presented in this thesis is to extend the current knowledge on risk factors for cognitive decline in type 2 diabetes. For this purpose, risk factor associations with trajectories of late-life cognitive change will be investigated in a population consisting exclusively of diabetes patients. Data from the baseline, the year 1 and year 4 follow-up waves of the prospective Edinburgh Type 2 Diabetes Study (ET2DS) of community-dwelling patients with type 2 diabetes will be used to statistically test associations between baseline risk factors and cognition. The main outcome of interest is the rate of cognitive change between baseline and year 4 of the study. Additionally, cross-sectional associations with estimated peak pre-morbid ability, with level of cognitive function measured at year 4, with estimated lifetime cognitive change between pre-morbid ability and year 4, and with presence of diagnosed dementia will be explored. Both specific cognitive domains and global cognitive function will be assessed. In the first part of the thesis, the relationship of the main risk factors under investigation, including one which is unique to diabetes (severe hypoglycaemia) and one which is linked to the condition but (at lower prevalence) also occurs in non-diabetic populations (macrovascular disease), with cognitive outcome will be determined in detail. Multivariable adjustment will be applied with the aim to evaluate potential mechanisms underlying any statistically significant associations. In the second part of the thesis, exploratory analyses will consider and compare a wider range of vascular and metabolic risk factors in their ability to predict cognitive outcome in slightly less detail. Finally, the usefulness of a summary 'allostatic load' measure derived from a number of individual risk factors in its relationship with cognitive decline will be determined.

3.3.2 Objectives

1. To determine the associations of baseline macrovascular disease with: (i) estimated peak pre-morbid cognitive ability, with (ii) level of late-life cognitive ability measured at follow-up, with (iii) four-year late-life cognitive change between baseline and follow-up, and with (iv) estimated lifetime cognitive change between estimated peak pre-morbid ability and late-life ability in a sample of older people with type 2 diabetes.
2. To determine the potential bidirectional relationship between hypoglycaemia and cognitive function in the same sample by associating a baseline history of severe hypoglycaemia and severe hypoglycaemia during follow-up: with (i) estimated peak pre-morbid cognitive ability, with (ii) level of late-life cognitive ability, with (iii) four-year cognitive change and with estimated lifetime cognitive change, and by (iv) associating cognitive function at baseline with the risk of severe hypoglycaemia during follow-up.
3. To compare the predictive ability of a wider range of risk factors measured at baseline: with respect to (i) level of late-life cognitive ability at follow-up, with (ii) four-year cognitive change, and with (iii) estimated lifetime cognitive change. In addition to macrovascular disease and severe hypoglycaemia, risk factors considered in this part of the analyses will include:
   - (i) Conventional cardiovascular risk factors
   - (ii) Microvascular disease
   - (iii) Inflammation
   - (iv) Hyperglycaemia
   - (v) Glucocorticoids

4. To determine the usefulness of an ‘allostatic load’ measure constructed from a number of baseline risk factors and (if applicable) its prediction of late-life cognitive outcome.

In order to achieve these objectives, a series of tasks will be undertaken:

1. Test participants in the ET2DS for cognitive ability, four years after they were recruited into the study, and analyse change in cognitive function over the four-year period.
2. Assess the representativeness in terms of demographic, clinical and cognitive characteristics of ET2DS participants who returned to year 4 follow-up.
3. Collect data on the incidence of dementia in the ET2DS and repeat key analyses with exclusion of subjects with dementia in order to evaluate the independence of overall findings from a contribution by dementia. Analyse statistically the associations of pre-selected risk factors, measured at baseline or during follow-up, with: (i) estimated pre-morbid ability, with (ii) level of cognitive function at year 4, with (iii) four-year cognitive change, and with (iv) estimated lifetime cognitive change.
4. Determine whether or not ‘allostatic load’ derived on the basis of a number of pre-selected risk factors is a useful summary measure, and, if this is found to be the case, to associate this measure with cognitive decline.

Chapter 4: Methods

This chapter describes the design of the Edinburgh Type 2 Diabetes Study (ET2DS) and the data relevant to this thesis which were collected at the baseline and year 1 waves of the study. These phases of data collection were completed before I joined the study. Data collection, data cleaning and derivation of variables are also described for the four-year follow-up phase of the study, in which I was integrally involved. The statistical analyses included in this thesis as well as their theoretical background are specified in the final sections of the chapter.

4.1 The Edinburgh Type 2 Diabetes Study: study design

The ET2DS was set up in 2006/2007 with funding from the Medical Research Council (MRC) and the primary objective to investigate associations between modifiable risk factors and cognitive decline, in order to inform the future development of strategies and therapeutic agents to protect cognitive function in type 2 diabetes (J. F. Price, Reynolds, et al., 2008). Additional outcomes of interest were fatty liver disease as well as micro- and macrovascular disease (J. F. Price, Reynolds, Frier, & Strachan, 2008).

A large sample of older patients with type 2 diabetes who were living in the Lothian area of Scotland, UK, were recruited, extensively phenotyped and followed up over several waves. The study is a population-based cohort study investigating exposure-outcome relations in a population defined by geographic boundaries and group membership in an observational fashion (Szklo, 1998). Although retrospective cohort studies are possible, the ET2DS has the preferred prospective design, which allows the addition and exclusion of measurements during follow-up and enables observation of incidence and prognosis of disease as well as continuous change in outcomes. Although this type of study does not allow conclusions regarding causality underlying any associations, the temporal relationship between exposure and outcome may be inferred when both occur at different time points. Ultimately, conclusions drawn on the basis of prospective investigations may be more reliable at least when compared with cross-sectional or case-control studies (Mann, 2003). This may provide important information for the design of future studies aimed at identifying causality in associations, although the view that evidence from this type of study is of a quality *comparable* to that from 'gold-standard' randomised controlled trials (Micha & Mozaffarian, 2010) appears exaggerated.

A majority of cohort studies of cognitive ageing, such as the Framingham Study (Akomolafe, et al., 2006) or the Rancho Bernardo Study (Kanaya, et al., 2004), recruit large samples from older general populations and investigate type 2 diabetes as a secondary predictor of cognitive outcome. Rarely are individual differences in a range of different risk factors associated with cognitive outcome in populations consisting exclusively of older patients with type 2 diabetes. The ET2DS follows precisely this approach. By targeting risk factors within this high-risk group will enable an investigation of the relationship of individual differences in risk factor prevalence or severity with cognitive decline and will thereby exceed simple description of diabetes-associated cognitive decline by allowing the investigation of the specific underlying causes of these well-established associations. The study therefore offers a novel contribution to the research literature on diabetes-associated cognitive decline. To date, two main waves have been completed.

4.2 Study population

Details of recruitment have been described in the study protocol (J. F. Price, Reynolds, Mitchell, et al., 2008). Participants were recruited from the Lothian Diabetes Register (LDR) which captures almost all patients with diabetes diagnosed according to WHO criteria living in the Lothian region of Scotland (n=~20 000). 5454 individuals on the LDR who were between 60 and 74 years of age on 1st August 2006 were randomly selected by sex and five-year age bands and were contacted by post; 1252 individuals replied and expressed interest. The baseline clinic in 2006/2007 was subsequently attended by 1077 subjects. Participants with the entire spectrum of severity of diabetes were recruited: mode of treatment ranged from diet-controlled to insulin-controlled. Individuals with poor corrected visual acuity (distance vision <6/36 or unable to read large print text) were excluded from the study, because some cognitive tests were paper-based. Non-native speakers of English were also excluded. This limitation was applied due to language requirements of cognitive tests, but is unlikely to restrict generalisation of the study results to the general Scottish population, considering that only around 3% of Scots are born outside of the UK and therefore likely to be non-native speakers (General Register Office for Scotland, 2008).

Following completion of the baseline clinic, medical records were assessed by the study team in order to exclude any patients erroneously recorded on the LDR as having type 2 diabetes. Type 2 diabetes status was taken as confirmed if a patient was treated with oral anti-diabetic agents and/or insulin, or had HbA1c levels >6.5%. Individuals who were not receiving treatment, had HbA1c ≤6.5%, had started on insulin within one year of diagnosis, had self-reported pancreatic surgery or disease, or who were on insulin-treatment and aged <35 years at the time of diagnosis were reviewed by a consultant diabetologist.

The overall temporal structure and subject participation of the ET2DS are shown in Figure 4.1. Of all participants attending the baseline clinic in 2006/2007 (n=1077), subjects with questionable type 2 diabetes diagnosis or a history of pancreatic disease (n=7) and those who were not cognitively tested or physically examined due to refusal or physical disability (n=4) were excluded from the study. This resulted in a total baseline population of 1066. Around one year after the baseline clinic, all 1066 participants were invited to return for further assessments, predominantly of liver abnormalities. 939 individuals attended this clinic and 898 consenting subjects were there enrolled in a 6-month survey of severe hypoglycaemia (SH). In the time period between baseline and year 4, 88 (8.3%) participants died (mortality was the cause of 37.4% of attrition between baseline and year 4), nine (0.8%) declined further attendance and 26 (2.4%) were deemed unfit to continue with the study. Reasons for withdrawal or non-attendance included poor health (including dementia), responsibilities as a carer, and other personal reasons. Following the baseline clinic, one participant was identified as failing to meet WHO diagnostic criteria for diabetes. The remaining 943 participants of the total baseline population (n=1066) were invited for the year 4 follow-up. In 2010/2011, and 831 participants attended the clinic; 828 of these provided cognitive data. Physical examination procedures and cognitive test batteries were largely identical at baseline and year 4, with additional assessment for cIMT undertaken at the year 1 clinic. Details of these examinations are described below.

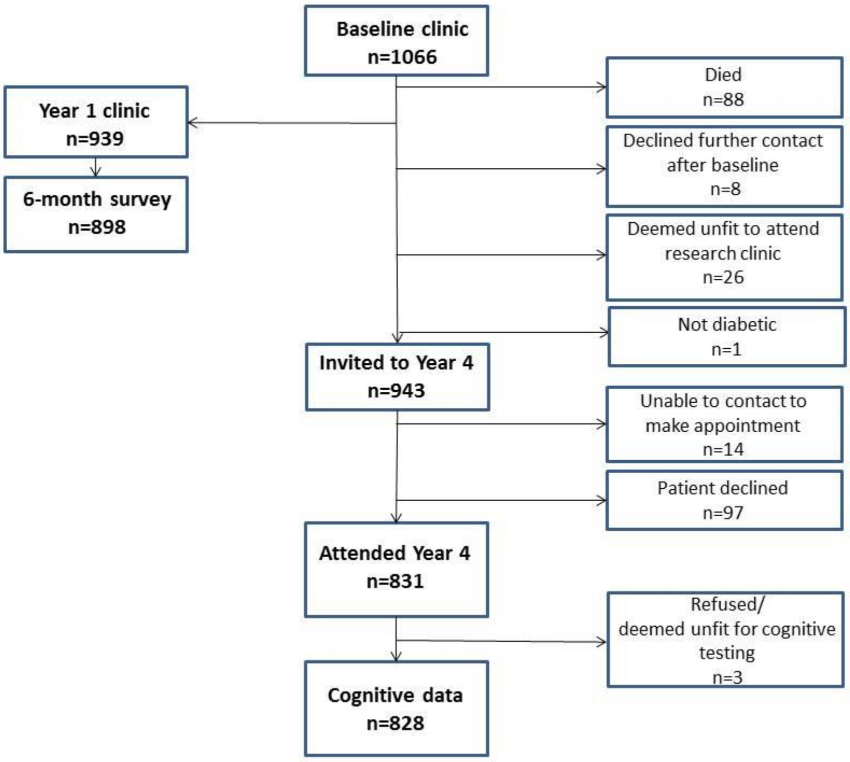

Figure 4.1: Subject participation in the ET2DS

4.3 Ethical approval

The study had full ethical approval from the Lothian Medical Research Ethics Committee and assessments complied with the Declaration of Helsinki. Informed consent was obtained from all participants at each point of data collection.

4.4 Physical and cognitive examination

Clinical examination was carried out by four to six members of the research team on each weekday morning between August 2006 and September 2007 (at baseline) and between May 2010 and May 2011 (at year 4) in the Wellcome Trust Clinical Research Facility at the Western General Hospital, Edinburgh. A taxi service to the clinic was offered; appointments affected by severe weather conditions were rescheduled. Patients were fasted overnight and instructed to collect morning urine samples. Physical and cognitive assessments were completed between morning and noon, although the specific order of examinations varied. All members of the research team were trained in their respective assessments.

4.4.1 Questionnaires

Baseline questionnaires

Baseline questionnaires collected self-reported information on educational attainment, occupation, current employment status of the participant and their spouse, marital status, ethnicity, years since diagnosis of diabetes and current diabetes treatment. Information on previous doctor's diagnosis of cardiovascular disease, including myocardial infarction and angina, and history of surgical operations were also self-reported. Deprivation was determined by the Scottish Index of Multiple Deprivation (SIMD) determined on the basis of participants' postcodes; this was represented in quintiles (first quintile- most deprived; fifth quintile- least deprived).

Severe hypoglycaemia (SH) was defined as an episode which required the intervention by another person for recovery. In order to ascertain participants' lifetime history of SH, an initial question asked if the participant had ever experienced an episode of low blood glucose which required treatment by another person, for instance using a sugary drink or glucagon. It was noted that such episodes are commonly termed 'hypoglycaemia'. Possible responses to the item were 'yes', 'no' and 'I don't know'. A positive response to the item was followed-up by questions on the number of such episodes that had been experienced in total over the course of their lifetime (possible responses '1-2', '3-4', '5 or over'), and in the year prior to the clinic visit (possible responses '1-2', '3-4', '5 or over'). Uncertainty in subjects who selected 'I don't know' was followed up and resolved by research staff if possible.

The Edinburgh Claudication Questionnaire (Leng & Fowkes, 1992) assessed presence of intermittent claudication, which for the purpose of this thesis is referred to as 'PAD'. Administration of the World Health Organisation (WHO) Chest Pain Questionnaire aided the identification of individuals with cardiovascular disease. Alcohol intake over the past week was self-reported (number of spirits/wines on each day), as was a doctor's diagnosis of alcohol abuse. Current smoking habits (number of cigarettes/cigars or ounces of tobacco per day) and history of smoking for ex-smokers (years/months since cessation) were ascertained.

Six-month survey of severe hypoglycaemia

Of all subjects attending the year 1 clinic (n=939), 898 agreed to participate in a 6-month survey of severe hypoglycaemia (SH) carried out immediately after year 1 clinic visit. The questionnaire was based on the Edinburgh Hypoglycaemia Scale (Deary et al., 1993) and included items on symptoms, date and time of any hypoglycaemic episode, loss of consciousness, details on help from another person, details of treatment and blood glucose values if measured (Appendix H). Subjects self-completed the questionnaires and returned one every two months over a six month period (total of three questionnaires per person) irrespective of whether they had experienced SH or not. Participants who reported SH and those who failed to return a given questionnaire were contacted by telephone by a clinically qualified member of the research team in order to ensure data accuracy and completeness.

Year 4 questionnaire

Year 4 self-completion questionnaires collected up-dated information on marital status, current occupation of participants and his/her spouse, diabetes history and treatment, cardiovascular disease, alcohol intake over the past week, history of alcohol abuse, and history of smoking, including number of cigarettes/cigars or ounces of tobacco smoked per day and months/years since cessation. The questionnaire also included items on whether or not subjects had experienced SH during the past four years (i.e., since baseline) and during the past year. Responses again were 'yes', 'no' and 'I don't know'. Positive responses were followed by items on the number of episodes which had been experienced during the past four years (possible responses '1-2', '3-4', '5 or over'), and during the year prior to this clinic visit (possible responses '1-2', '3-4', '5 or over'), and participants who expressed uncertainty were followed up by the research team where possible. Again, the Edinburgh Claudication Questionnaire (Leng & Fowkes, 1992) determined possible intermittent claudication, and the World Health Organisation's (WHO) Chest Pain

Questionnaire was administered to aid identification of individuals with cardiovascular disease.

<u>Hospital Anxiety and Depression Scale (HADS)</u>

At both baseline and four-year follow-up, two subscales of the self-administered Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) measured anxiety (HADS-A) and depression (HADS-D). On each, participants self-report symptoms of depression and anxiety over the last few days, respectively, by selecting 'most of the time', 'a lot of the time', 'from time to time' and 'not at all' in response to statements describing symptoms (e.g., 'I feel tense or wound up', HADS-A). Maximum score is 21 for each scale, minimum scores are zero. High scores indicate a high number of symptoms. A recent systematic review established high validity for the HADS in general and in patient populations, as well as a two-factor structure consistent with HADS-A and HADS-D (Bjelland, Dahl, Haug, & Neckelmann, 2002). The subscales also have high sensitivity and specificity (Bjelland, et al., 2002; Olssøn, Mykletun, & Dahl, 2005), and have further been shown to have high reliability and a robust factor structure in diabetic populations (Reddy, Philpot, Ford, & Dunbar, 2010). Scores may be used as continuous measures or with cut-off ≥8 to indicate suspected clinical depression and anxiety, respectively. Both applications are common in the literature. Copies of all questionnaires can be found in Appendix H.

4.4.2 Physical assessment

<u>Physical examination</u>

At both baseline and four-year follow-up, systolic blood pressure was taken in two readings from left and right brachial, posterior tibial and dorsalis pedis arteries using Doppler in a supine position and following a five minute rest unless this was undesirable or impossible. Additionally, systolic and diastolic blood pressure was taken from the right arm. Height to the nearest millimeter, body weight to the nearest 0.1 kg, twelve-lead ECG, waist circumference and hip circumference were recorded. For waist and hip circumference, two readings were carried out to the nearest 0.5 cm and averaged. Duplication of all recorded values over a pre-specified period of time during the clinics determined that inter-individual differences between testers in the precision of measurement were acceptably low at both baseline and at year 4. For all participants, ECG was rated for possible cardiovascular disease by a single rater. In addition to these assessments, distance and near vision and % body fat content were measured at baseline only. Three readings of body fat content were averaged.

Blood and urine assessments

At baseline, fasting blood samples were taken for measurement of low-density lipoprotein (LDL), high-density lipoprotein (HDL), inflammatory markers (c-reactive protein, CRP; interleukin-6, IL-6; tumor necrosis factor-alpha, TNF-α, fibrinogen), HbA1c, plasma glucose, red and white cell counts, platelet counts, creatinine and cortisol. Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations were determined using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK) calibrated using the manufacturer's reagents. Manufacturer's controls were used with limits of acceptability defined by the manufacturer. Low control coefficient of variation (CV) was 6.7% and high control CV was 4.9%. At year 4, fasting blood samples determined fasting blood glucose, HbA1c, lipids, apolipoprotein A1 and B, creatine and cortisol. All blood and urine samples were frozen at -80º C for storage.

Diabetic retinopathy (DR)

Two to three weeks following their initial baseline visit to the clinic, 1046 patients of the total baseline population returned for digital retinal photography. Seven-field non-stereoscopic colour photographs were taken of both eyes using a high resolution digital retinal camera and were graded independently by two trained optometrists. Grading was carried out according to a scale used by the Early Treatment Diabetic Retinopathy Study (ETDRS) (1991). Scores ranged between 10 (no retinopathy) and 81 (advanced proliferative retinopathy), and were categorised into 'no DR', 'mild DR' and 'moderate/severe DR'. Discrepancies between the graders was resolved by discussion, and if unresolved were reviewed by an ophthalmologist. For each participant, severity of retinopathy in the eye more severe affected was used for all analyses. Data from two participants was excluded due to low image quality, so that overall retinopathy data was available for 1044 participants. A detailed description of the photography procedure has been published previously (Ding, et al., 2010).

Carotid intima-media thickness

Approximately one year later, all baseline participants (n=1066) were invited to the year 1 clinic. 939 participants attended the clinic and there had carotid intima-media thickness (cIMT) examined, a procedure which was repeated again at four-year follow-up. At both year 1 and year 4, carotid IMT was visualised bilaterally in three separate images of the common carotid artery, one to two cm below the bifurcation and in areas free of plaque using a Sonoline Elegra Ultrasound Imaging System (Siemens Medical Systems Inc, Washington). Mean cIMTs were calculated for left and right carotid arteries and the larger of the two values was used for analyses.

4.4.3 Record linkage

Participants gave full written consent to allow access to their medical records. Historical data on any blood pressure readings and HbA1c collected between 1988 and September 2007 was collected from the Lothian Diabetes Register (LDR) for all participants. At baseline and again at year 4, records of acute hospital admissions on the SMR01 scheme of the Information and Services Division of NHS Scotland were also consulted for the entire baseline population. These records included information on dates of hospital admission, medical conditions, operations and cause of death from 1981 up to September 2007 (end of baseline clinic) and from September 2007 (end of baseline clinic) to July 2011) (end of year 4 clinic). Any ICD-9 and ICD-10 codes for cardiovascular disease, cerebrovascular disease or dementia were identified from this database and used in combination with self-report data and findings from the chest pain questionnaire and the ECG to identify individuals with a history of myocardial infarction, angina, stroke, transient ischaemic attacks or dementia according to pre-specified criteria described below.

4.4.4 Cognitive assessment

With exception of the Deary-Liewald reaction time task which was only administered at year 4, the same battery of cognitive tests was used at baseline and follow-up. Seven neuropsychological tests of fluid-type cognitive ability, a test screening for dementia and a test estimating crystallised-type pre-morbid ability were administered in a single session lasting approximately one hour. Cognitive assessment was performed following food intake and measurement of plasma glucose levels. Individuals with levels <4 mmol/L were not cognitively tested but first offered food. Following a brief period of rest, blood glucose was re-tested to ensure that values exceeded 4 mmol/L. Prior to the clinic appointment, participants had been reminded to bring their reading glasses, and testers ensured that these were worn during the testing session if possible. Subjects who did not perform any of the cognitive tests at baseline due to either refusal, physical disability or because they were deemed unfit (n=4) were excluded from the baseline population of the ET2DS and were not invited to return for any subsequent waves of the study. Participants without any cognitive data at year 4 (n=3) were not excluded. The seven tests of fluid ability tapped a variety of cognitive domains, and were selected on the basis of common use in the literature, evidence of validity and/or susceptibility to decline in diabetes. Within each cognitive testing session, a strict order of administration was followed where possible. Copies of some of the paper-based cognitive tests can be found in Appendix G.

Borkowski Verbal Fluency Test

Tests of verbal fluency load on executive function processes and involve the production of words from a phonemic or semantic category which are pre-determined by the tester (Klumpp & Deldin, 2010). The phonemic Borkowski Verbal Fluency Test (BVFT) (Borkowski, Benton, & Spreen, 1967) was used in the ET2DS. Subjects name as many words as possible beginning with C, F and L, excluding proper nouns, within 3 x 60 seconds. One point is given for each word with no upper limit in achievable scores. Performance relies on the maintenance of tasks sets, the initiation of multiple response alternatives and monitoring of responses to avoid repetition. Verbal fluency is affected by age (Salthouse, Atkinson, & Berish, 2003), mild cognitive impairment (Cunje, Molloy, Standish, & Lewis, 2007) and mood disorders (Klumpp & Deldin, 2010). Temporal and frontal areas, including Broca's speech area and the dorsolateral pre-frontal cortex, are recruited during performance (Amunts et al., 2004; Klumpp & Deldin, 2010; Libon et al., 2009). The validity of tests of verbal fluency to measure frontal lobe and executive function was shown by a study which reported correlations of verbal fluency with relative/carer-reported everyday executive function (Burgess, Alderman, Evans, Emslie, & Wilson, 1998).

However, verbal fluency is also strongly related to level of education (Tombaugh, Kozak, & Rees, 1999; van der Elst, van Boxtel, Van Breukelen, & Jolles, 2006) and to prior experience with crosswords or other word games (Phillips, 1997 cited in (Crawford, Bryan, Luszcz, Obonsawin, & Stewart, 2000)).

Trail-Making-Test-B

The Trail-Making-Test-B (TMT-B) measures mental flexibility, processing speed, visual attention and executive function (Salthouse, et al., 2003). Participants are presented with an A4 sheet of paper with dots with numbers and letters and connect dots alternating between numbers and letters (e.g., A-1-B-2…). Following a short practice of seven connections, completion of the trial involving 26 connections is timed. Higher TMT-B scores indicate poorer performance. The test's high executive function demands caused by inhibition of prepotent responses (A-B-C…) are reflected in the involvement of frontal areas during task performance (O'Sullivan et al., 2001; Oosterman et al., 2010). The TMT-B is well-validated (Burgess, et al., 1998), is sensitive to age effects as well as cognitive impairment and impaired activities of daily living (ADL) (Cook et al., 2002; Mitrushina, Boone, Razani, & D'Elia, 2005) and may be immune to mood disorders (Misdraji & Gass, 2010). However, due to the wide range of cognitive functions involved in TMT-B performance, poor performance is a non-specific finding. Education and graphomotor skills also affect performance (Misdraji & Gass, 2010; Mitrushina, et al., 2005). Indeed subjects in the ET2DS occasionally experienced physical difficulty. A difference score between the TMT-B and a simpler version (TMT-A; connection of dots 1-2-3…) helps to eliminate effects of graphomotor skills, but TMT-A in practice is rarely administered, including in the ET2DS. Although cut-points may be applied (Kilander, Nyman, Boberg, Hansson, & Lithell, 1998), the present analyses will use all TMT-B measurements in order to reflect the entire breath of performance in the population.

Wechsler Memory Scale 3rd Edition

Two subtests of the Wechsler Memory Scale 3rd Edition (WMS-III) (Wechsler, 1987), which is widely used in clinical settings, were included.

Logical Memory

In the Logical Memory (LM) subtest of verbal declarative memory, participants listen to a short story with 25 story units and recall the story immediately after presentation and again following a delay of around 40 minutes. One point is given for each correctly recalled unit. As immediate and delayed recall of the task correlate highly (Tulsky, Ivnik, Price, & Wilkins, 2003), both scores typically correlate highly and may be summed to represent overall verbal memory (maximum score 25). Performance appears to involve prefrontal and parietal areas (Simensky & Abeles, 2002). Previous studies have shown age-related declines in verbal memory and in Logical Memory scores in particular (D. K. Johnson, Storandt, & Balota, 2003; L. Price, Said, & Haaland, 2004; Simensky & Abeles, 2002), with some evidence for differential effects on immediate and delayed recall (D. K. Johnson, et al., 2003). Performance on the test may be able to dissociate normative cognitive ageing, MCI and dementia (Cunje, et al., 2007).

Faces

The Faces subtest assessed nonverbal memory. Twenty-four pictures of faces are first exposed, each for around two seconds. Subjects then identify the familiar faces out of a sample of 48, which include 24 novel pictures, through verbal responses 'yes' (I have seen this face before) and 'no' (I have not seen this face before). Instructions specify that for each item the initial best guess should be verbalised. The procedure is repeated both immediately following presentation of the target stimuli (immediate recall), and with a delay of around 20 minutes (delayed recall). On both occasions, maximum score is 48. Scores may be summed to obtain a composite score of non-verbal memory (maximum score 96). Due to high complexity and intra-class similarity, faces are somewhat 'special' when compared with other visual stimuli (Werheid & Clare, 2007). The Faces test is therefore immune to strategies such as verbal encoding which would mask subjects' actual nonverbal memory abilities. Despite this advantage, the validity of the test has been questioned on the basis of low correlations with other tests of visual memory, which may be caused by a unique hard-wired memory for this 'special' stimulus (Tulsky, et al., 2003). Moreover, women may have advantages in memory for faces over men (Bengner et al., 2006; Weirich, Hoffman, Meissner, Heinz, & Bengner, 2011).

Wechsler Adult Intelligence Scale 3rd Edition

Subtests of the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) (Wechsler, 1997) were included in the cognitive test battery. The WAIS-III is a well-validated group of measures shown to have high reliability and high loadings on the general ability factor *g* (Kaufman, 2000; Lichtenberger & Kaufman, 2009; Shelton, Elliott, Hill, Calamia, & Gouvier, 2009).

Digit Symbol Coding

The paper-and-pencil based Digit Symbol Coding (DSC) subtest of the WAIS-III assesses speed of information processing. A table is presented at the top of an A4 page linking specific arbitrary symbols to digits 1 to 9. Rows with digits but missing symbols are printed below the table, and participants are instructed to match symbols to their respective digits. Maximum score is 133. A time limit of 120 seconds minimises ceiling effects. Test performance involves visual search, attention and constant updating of working memory. Recruitment of frontal, prefrontal and parietal areas reflect the wide variety of processing demands of the task (Usui et al., 2009). Digit Symbol Coding performance declines with age but is relatively unaffected by education (Hoyer, Stawski, Wasylyshyn, & Verhaeghen, 2004). However in the light of the advanced age of the ET2DS population influences of visual acuity and motor coordination on performance (Lichtenberger & Kaufman, 2009) must be considered. Individual differences in incidental learning of the digit-symbol combinations may affect results (Joy, Kaplan, & Fein, 2003) although opposing findings have also been reported (Erber, Botwinick, & Storandt, 1981).

Letter Number Sequencing

In the Letter Number Sequencing (LNS) subtest - which is used to assess working memory-participants are verbally presented with sequences of numbers and letters at a rate of around one item per second. The sequence is then mentally manipulated into alphabetic and numeric order and is verbalised to the tester. Eight blocks of sequences (each with three trials) have increasing length. The initial block includes three numbers and letters and this increases by one number/letter with each new block. Successful completion of each trial counts as one score. Maximum score is 21. Performance is terminated following unsuccessful completion of all three trials within a given block. Working memory is defined as a temporary storage system responsible for information processing (Baddeley, 1981). According to one popular model (Baddeley & Hitch, 1974 cited in (Baddeley, 1981)), it comprises of a central executive, an articulatory loop rehearsing verbal information, and a visuo-spatial scratchpad which enables the visualisation of processed content. Consequently, attentional abilities affect Letter-Number Sequencing performance. The test has been shown to have high construct validity as a test of working memory (Shelton, et al., 2009); evidence of right hemisphere activation during performance (Haut, Kuwubara, Leach, & Arias, 2000) could potentially reflect involvement of the hypothesised visuo-spatial scratchpad.

Matrix Reasoning

The Matrix Reasoning subtest (MR) of non-verbal reasoning is presented in a 28-page booklet. Several pictures are printed on each page and subjects identify the one picture out of five choices which best completes a geometric sequence. Following two practice items, 26 trial items are completed, with a maximum score of 26. Performance is terminated when choices are incorrect on either four consecutive or four out of five consecutive items. The test relies on visual acuity, visuo-spatial construction and the ability to use trial and error, and assesses non-verbal and abstract perceptual reasoning abilities with high loading on the general ability factor *g* (Salthouse, et al., 2003). Reliance on working memory resources and executive function is evident in activation of pre-frontal areas during abstract reasoning tasks (V. Prabhakaran, Smith, Desmond, Glover, & Gabrieli, 1997) as well as in poor reasoning abilities in patients with frontal atrophy (Yoshiura et al., 2011). Abstract reasoning declines with age and is reduced in dementia (Cronin-Golomb, Rho, Corkin, & Growdon, 1987). The Matrix Reasoning test has high face validity and correlations with Raven's Progressive Matrices of abstract reasoning indicate high construct validity (Kohutek, 1999). Due to reliance on concentration skills and motivation, reasoning performance may be vulnerable to mood disorders. The use of the test is further complicated by the fact that although the tester is informed that performance should not exceed 15 minutes, the test is not strictly timed and time is not mentioned during instructions. Evidence suggests that the resulting confusion over instructions may affect subjects' reasoning test scores (Knight, 2003).

Deary-Liewald reaction time task

The Deary-Liewald reaction time task (Figure 4.2), which measures processing speed, was administered only at the year 4 clinic. It was performed on a conventional computer at the end of each testing session in order to ensure that performance on other cognitive tests was unaffected by task-associated psychological distress. The test measures simple reaction time (SRT) and four-choice reaction time (CRT) in two paradigms: in the SRT, participants respond to the appearance of a stimulus on the computer screen by pressing the spacebar on the keyboard as quickly as possible. Performance does not involve evaluation of content other than presence or absence of the stimulus (W. Johnson & Deary, 2011). In the CRT, a target stimulus is detected in one of four possible squares on the screen. Responses are made by pressing a key corresponding to the square ('Z', 'X', 'comma', 'full stop'). Thus, for both the CRT and SRT, response times are the sum of the speed of processing of the stimulus, and the movement time required to carry out the response. For each task, subjects initially performed eight practice trials, before completing 20 trials in the SRT and 40 trials in the CRT. Inter-stimulus intervals ranged between 1000 and 3000 milliseconds for both tasks. Latency between stimulus presentation and response was measured and recorded automatically by the computer program for each trial. In addition to average latency of each participant, their intra-individual variability was also recorded.

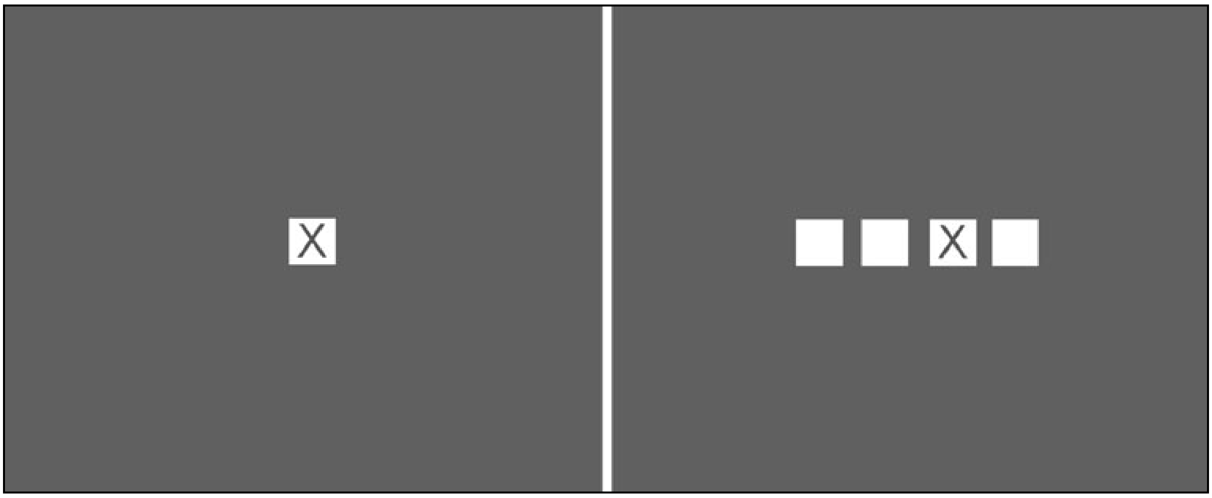

Figure 4.2: Screenshot of the Deary-Liewald task for the SRT (left) and CRT (right), reproduced from Deary et al. (2011)

The Deary-Liewald task was recently validated against other measures of reaction time across age groups (Deary, et al., 2011). Its use offers great advantages over traditional paper-and-pencil tasks, including the output of absolute values (rather than scores on arbitrary scales), a high precision in the recording of responses, as well as quickness and consistency in task administration. This allows completion of a large number of trials for each participant and performance is immune to experimenter expectancy. However, task performance may be affected by subjects' proficiency in computer use, which may linked to crystallised-type ability (Nair, Czaja, & Sharit, 2007). Sex differences in reaction time are also well-established (Roivainen, 2011).

Mill Hill Vocabulary Scale

The Junior and Senior form A of the Mill Hill Vocabulary Scale (MHVS) (Raven, Raven, & Court, 1998) measures crystallised-type intelligence by estimating best-ever pre-morbid cognitive ability. Throughout this thesis, scores on the MHVS will be referred to as pre-morbid cognitive ability despite reflecting estimation and not measurement. Participants are presented with 43 groups of words, and instructed to identify the one word out of a choice of six which is a synonym of a target word printed above each group of words. One point is obtained for each correctly identified word (maximum score 43). The MHVS may be preferable over self-reported education as an estimate of pre-morbid ability, because education is affected by circumstantial factors, particularly in currently older populations. The National Adult Reading Test (NART), which involves the verbalisation of words with increasing difficulty, is often preferred over the MHVS to estimate peak pre-morbid ability on the basis of vocabulary, but in contrast to NART, the MHVS allows self-completion. In the light of the relatively large and time-intensive battery of cognitive tests and relatively large sample size of the ET2DS, the MHVS was therefore seen as advantageous. Its use is supported by observations of typically strong correlations of scores on the MHVS with scores on other estimates of peak pre-morbid ability including NART (O'Carroll & Gilleard, 1986; Yuspeh & Vanderploeg, 2000). For instance, in one early investigation of dementia patients and healthy older adults, the correlation coefficient of MHVS with NART was 0.69 (O'Carroll & Gilleard, 1986). Both tests also appear to be relatively immune to age-related declines or dementia (Deary, Whalley, & Crawford, 2004; McGurn et al., 2004), overall showing that both appear to be similarly valid representations of crystallised-type cognitive ability.

Mini-Mental-State-Examination

Due to a quick and easy administration, the Mini-Mental-State-Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) is a popular test of global cognitive ability and is used by clinicians and in the research literature alike. The test includes items on orientation to time and place, immediate and short-term memory, attention, calculation, language and praxis, and has overall good reliability, construct validity and specificity (Lancu & Olmer, 2006; Tombaugh & McIntyre, 1992). Maximum score is 30. Some studies, for instance the Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial (de Galan, et al., 2009) employ the MMSE as the main cognitive outcome variable in their analysis. Due to severe imprecision of this approach, as well as the neglect of some cognitive abilities such as processing speed which may be particularly susceptible to decline in diabetes (Cukierman, et al., 2005), the ET2DS instead relied on a battery of detailed neuropsychological tests on the basis of which a measure of global cognitive ability was derived. In line with its initial design, the MMSE only serves as a screening tool for cognitive impairment. Scores <24 are commonly used to indicate cognitive impairment (Lancu & Olmer, 2006). However neuropsychological follow-up is essential; the cut-point alone appears to have low reliability and validity (O'Connor et al., 1989). For instance, in two studies only 40% and 55% of subjects with MMSE<24 were eventually identified as suffering from dementia respectively (O'Connor, et al., 1989; Truelsen, Thudium, & Grønbæk, 2002).

Items on the MMSE also vary between different English-language versions, complicating a cross-study comparison of scores and the use of a cut-point to indicate cognitive impairment. For instance, versions vary in the specific words to be recalled following a brief interruption (e.g., ‘lemon’, ‘key’, ‘ball’). Some also include an item of serial sevens (counting down from 100 in steps of seven) (Chatfield, Matthews, Brayne, & Study, 2007), whereas other versions, including the one used in the ET2DS, replace this with backwards spelling of the word ‘world’. If a subject is unable to complete this item, five points (17% of total scores) are lost. Indeed, some participants of the ET2DS who self-reported being dyslexic (and who may have been illiterate) were unable to spell the word at all despite apparent good overall cognitive ability. In line with this observation, MMSE scores have been linked to education as well as cultural and socioeconomic backgrounds (Lancu & Olmer, 2006; Tombaugh & McIntyre, 1992). The cut-point indicative of cognitive impairment may be changed according to such factors (Bachman et al., 1992), but this is rarely applied in practice. Sex differences in MMSE scores have also been reported (de Galan, et al., 2009), and sensitivity and specificity of the test may be affected by mood disorders (Milian et al., 2012; Rajji et al., 2009). Finally, the test is unable to record changes in severity of dementia (Lancu & Olmer, 2006), although this does not apply to its use in the ET2DS.

Summary of cognitive test battery

A summary of the cognitive tests and their respective measured cognitive domains is provided in Figure 4.3 with the aim to provide the reader with a rough guide to the cognitive test battery used by the ET2DS and reported throughout this thesis. Note that the list of specific cognitive abilities assigned to each test is not exhaustive. For instance, visual attention also contributes to the performance of the Trail-Making Test-B. The MMSE is not included in the diagram, because it was used only as a screening instrument. Finally, a summary of the abbreviations for cognitive tests used throughout this thesis is shown in Table 4.1.

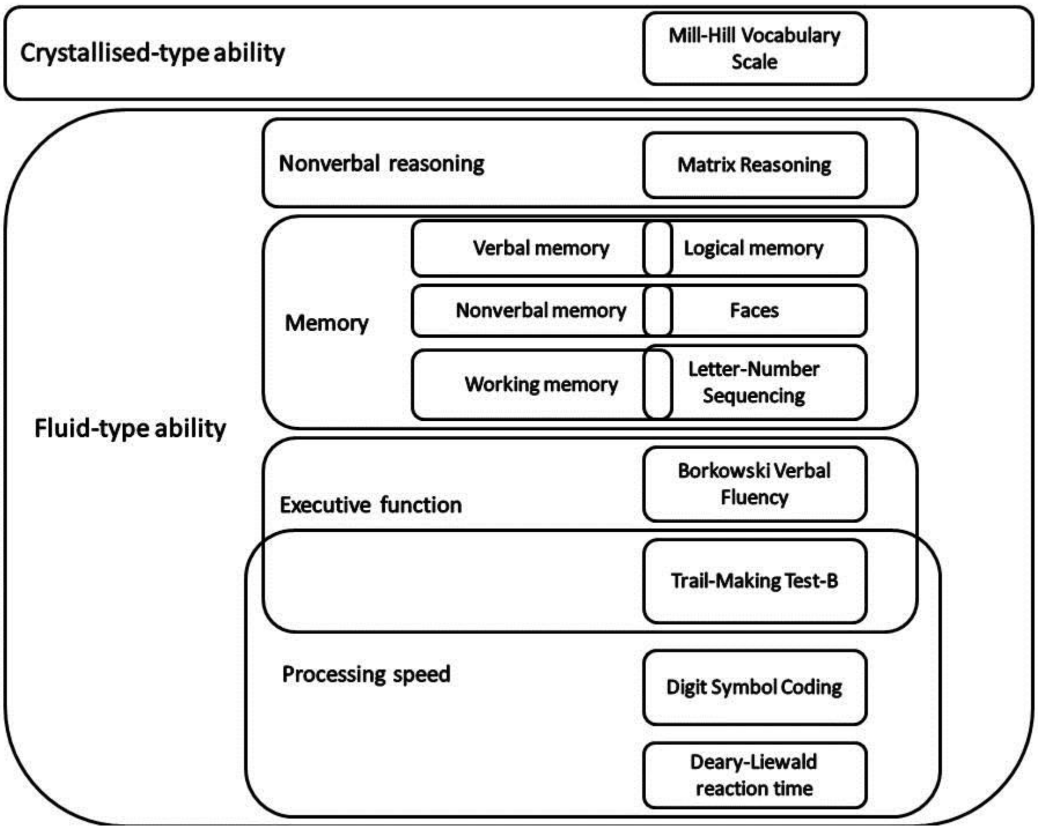

Figure 4.3: Cognitive tests used in the ET2DS. With exception of the Deary-Liewald reaction time test, all were administered at baseline and again at year 4

Table 4.1: List of abbreviations for cognitive tests

MHVS	Mill-Hill Vocabulary Scale
MMSE	Mini-Mental State Examination
LM	Logical Memory
Faces	Faces
MR	Matrix Reasoning
DSC	Digit Symbol Coding
TMT-B	Trail-Making-Test B
LNS	Letter Number Sequencing
BVFT	Borkowski Verbal Fluency Test

4.4.5 Dementia

The participants of the ET2DS live independently in the community, were required to schedule appointments and attended the research clinic(s). A majority is therefore assumed to be cognitively healthy. The identification of individuals with dementia is important nonetheless, because cognitive impairment is the end-point of age-related cognitive decline and even a few cases could skew the study's results. Although in practice dementia is only diagnosed following neuropsychological interview by a clinician, suspected cases (in both attenders and non-attenders of year 4) were identified in the ET2DS using information from a number of different available sources. These included MMSE performance at baseline and year 4, record linkage to hospital data, assessment of death records, self/relative report of dementia, self-reported use of medication for dementia, local old age psychiatry records and communication with patients' GPs. Specific findings from each of these sources are summarised in Appendix A. Because information on the time of diagnosis was not always available, and because disease onset may precede formal diagnosis by many years, no distinction was made between dementia that was prevalent at baseline and four-year incident dementia. The following criteria, which had been developed in co-operation with a consultant old age psychiatrist, were applied to identify participants who were likely to be suffering from dementia. It is understood that this method does not represent a substitute for neuropsychological assessment.

Either: 2 of (1) (2) (3) (4) (6)

or (5) plus 1 of (1) (2) (3) (4) (7)

(1) On medication for dementia (self-reported or GP reported use of donepezil, rivastigmine, galantamine, memantine)

(2) Hospital discharge code for dementia (ISD data) (ICD-10 codes F00, F01, F02, F03, G30; FXX or GXX were additionally checked)

(3) GP report of psychiatrist diagnosis of dementia

(4) Psychiatrist diagnosis of dementia obtained from psychiatry/hospital notes (PiMS)

(5) Mini-Mental-State Examination score <24 at baseline or year 4 or missing at year 4

(6) Self-reported and/or relative-reported dementia

(7) Code for dementia on death certificate

On the basis of these criteria, 19 individuals with suspected dementia were identified. For nine, the year of diagnosis was available. For all of these except one (who was diagnosed in 2004) diagnosis was made after completion of the baseline clinic (between 2008 and 2011; see Appendix A), suggesting that overall the 19 cases predominantly reflect four-year incident rather than prevalent dementia. Participants with suspected dementia were less likely to attend the year 4 clinic (n=4; retention rate 21.1%) compared with the remaining population (n=827; retention rate 78.7%; $p<0.001$). Although all dementia cases were retained in the database for the analyses presented in this thesis, some of the final multivariable analyses will be repeated with their exclusion in order to show that overall findings of risk factor associations with the cognitive continuum are not driven by dementia. Findings from these analyses are presented in Appendix E.

4.4.6 Summary of data collection

The temporal pattern of data collection in the ET2DS is illustrated in Figure 4.4.

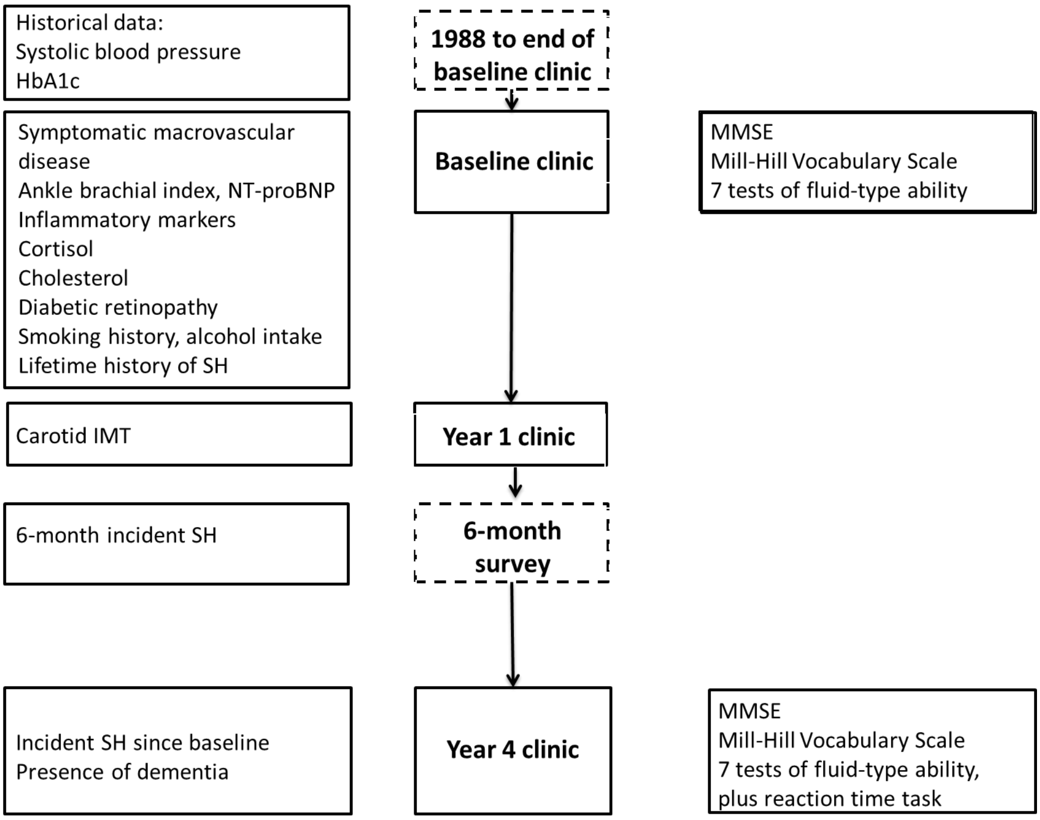

Figure 4.4: Data collection in the ET2DS

4.5 Data analysis

4.5.1 Cleaning of data

This section describes the process of data cleaning on the data from the four-year follow-up. Similar procedures had been carried out at baseline and year 1, as described in brief by Marioni et al. (2010).

Double data entry

Data was recorded in paper files for each participant and entered into a central Microsoft Access database securely stored at the University of Edinburgh. All cognitive data were entered on an on-going basis immediately following clinic appointments; entries were later checked by another member of the research team. Following completion of the year 4 data collection, the accuracy of information in the database was determined. For this purpose, data on cognitive and physical measurements as well as questionnaire data were re-entered into a separate database for a random group of participants (n=80; 9.6% of attenders). All entries were then checked against their original entries and any discrepancies were resolved. This procedure identified an error rate of 0.017% in the original database. When factors which could easily result in an error such as medication names were excluded, this rate attenuated to 0.004%.

Treatment of outliers

Following completion of all year 4 data entry, descriptive analyses were run on physical and cognitive measurements. Any 'implausible' physical outliers were resolved by the medically trained members of the research team through consultation of paper records and individual case assessment. For cognitive data, outliers were defined as scores >1.5 of the interquartile range of distributions. For each outlier, paper records were consulted and occasional data entry errors were resolved. Any outliers which were identical in database and paper records but were impossible were deleted and treated as missing. Any outliers which were identical in database and paper records but which were deemed plausible remained in the database and were to be excluded or included on an individual basis by the researchers who were using the data. In the analyses reported in this thesis, plausible outliers were retained with the aim to preserve statistical power, and because their analysis may be informative with respect to the research question. For the Deary-Liewald reaction time task, the computer automatically detected very long and very short response times (reflecting distraction or guessing prior to stimulus presentation, respectively), and excluded these from the final dataset. Response times between 150 and 1500 milliseconds were accepted as plausible in the SRT. For the CRT, acceptable response times ranged between 200 and 1500 milliseconds.

4.5.2 Use of clinical variables

Temporal pattern of clinical measurements

With exception of severe hypoglycaemia (data on which were used from baseline and from year 4 follow-up), only physical measurements and questionnaire data collected at baseline were used as exposure variables for the purpose of the analyses presented in this thesis. Because only one year passed between the baseline and the year 1 clinic, year 1 measurement of cIMT will be interpreted as equivalent to any baseline measurements.

Physical variables and blood measurements

Distribution of body fat mass was represented by the waist-hip-ratio (WHR) calculated from waist and hip circumferences. Ankle brachial pressure index (ABI) was determined by dividing the lowest of the lower limb blood pressures by the higher value of brachial pressure. Lower ABI values reflect higher degree of peripheral arterial disease. Because very high values may indicate arterial stiffness in diabetic populations (Potier, et al., 2011) data from participants with ABI >1.3 (n=26) was recoded as 'missing'. The ratio of low-density lipoprotein to high-density lipoprotein (LDL:HDL) measured dyslipidaemia.

Diabetic retinopathy

Severity of diabetic retinopathy (DR) was used as a binary variable ('DR', 'no DR') in descriptive analyses (to allow calculation of point bi-serial correlation coefficients), but was used in its original categorical form ('no DR', 'mild DR', 'moderate/severe DR') in all further analyses.

Historical data for blood pressure and HbA1c

Historical data collected since 1988 on the Lothian Diabetes Register (LDR) was used to derive average long-term HbA1c and systolic blood pressure measurements. For HbA1c, participants had between one and 59 readings available (median 13 readings; interquartile range 9 to 17). For systolic blood pressure, between one and 65 readings (median 19 readings; interquartile range 14 to 25) had been recorded. Readings for HbA1c and systolic blood pressure were summed and averaged for each participant. The resulting variables are referred to as 'historical HbA1c' and 'historical systolic blood pressure' throughout this thesis. The use of historical data has added to the validity of the present analyses, because HbA1c from blood samples collected at the clinic may be a poor indicator of long-term glycaemic control. Historical data on blood pressure avoided potential effects of clinic-associated psychological stressors, such as anticipation of cognitive testing, on measurements.

Questionnaire data

At baseline and year 4 follow-up, questionnaire data from individuals who expressed uncertainty over their experience of severe hypoglycaemia (which had not been resolved by research staff during data collection) were not used and so was treated as 'missing'. Self-reported smoking history was transformed to a continuous 'packyears' variable using a procedure which has been described in detail by a member of the research team involved in the baseline analysis of the ET2DS (Conaglen, 2009). In brief, packyears were calculated on the basis of self-reported number of cigarettes smoked per day. Smoking of cigars and pipes was relatively rare. When self-reported, the numbers of cigars/pipes were converted to cigarettes on the basis of estimated tobacco content (one cigar equivalent to four cigarettes; pipe smoking was self-reported in Oz. of tobacco) for each participant. The number of cigarettes was then transformed to number of packs (20 cigarettes in one pack) and multiplied by the number of years that the participant had smoked. One packyear is equivalent to 20 cigarettes smoked every day for one year, or 7300 grams of tobacco (Prignot, 1987, cited in (Conaglen, 2009)). Self-reported alcohol consumption during the week prior to clinic attendance was transformed to alcohol units/year. This continuous variable was used for the calculation of Pearson correlation coefficients, but all further analyses used the categorical variable. Categorisation was made to derive one group of abstainers, and quartiles of the subjects who consume alcohol (Conaglen, 2009) (Table 4.2).

Table 4.2: Minimum and maximum values of alcohol consumption in consumption groups

Alcohol consumption group	Units/year Minimum	Units/year Maximum
0 (abstainers)	0	0
1	14	53
2	54	314
3	319	817
4	857	5450

Presence of intermittent claudication was indicative of symptomatic peripheral arterial disease (PAD) and was determined from responses on the Edinburgh Claudication Questionnaire (Leng & Fowkes, 1992). Claudication codes were identified on the basis of the location of an X placed by participants on a diagram of their legs from a front and back viewpoint to indicate the location of pain. Grades were assigned according to responses on the item ‘Do you get [pain or discomfort in your legs] when you walk at an ordinary pace on the level?’. ‘Yes’ represented Grade 2; ‘no’ was defined as Grade 1. For the purpose of the present analyses, codes 1 to 7 in Table 4.3 indicated claudication; participants with code 8 were free of claudication.

Table 4.3: Coding from the Edinburgh Claudication Questionnaire

1 = Definite, Grade 1
2 = Definite, Grade 2
3 = Definite, Grade unknown
4 = Atypical, Grade 1
5 = Atypical, Grade 2
6 = Atypical, Grade unknown
7 = Possible claudication
8 = No evidence of claudication

Cardiovascular and cerebrovascular disease

The presence of cardiovascular and cerebrovascular disease was determined using a combination of self-report, record linkage and ECG data.

History of myocardial infarction was indicated when:

(i) 2 of the following 3 criteria were fulfilled:

Self-reported heart attack in questionnaire

Myocardial infarction indicated on WHO Chest Pain Questionnaire

Ischaemia indicated by ECG

Or

(ii) Both of 2 criteria were fulfilled:

Self-reported heart attack in questionnaire

ICD code consistent with myocardial infarction (I21-I23, I252)

Angina was indicated when

(i) 2 of the following 3 criteria were fulfilled:

Self-reported doctor-diagnosed angina or regular use of medication for angina
Angina indicated on WHO Chest Pain Questionnaire
Ischaemia indicated by ECG

Or

(ii) Both of 2 criteria were fulfilled:

Self-reported doctor-diagnosed angina or regular use of medication for angina
ICD code consistent with angina or other ischaemic heart disease (IHD) (I20, I24, I25)

History of cerebrovascular disease (stroke and/or TIA) was indicated when 2 of the following 3 criteria were fulfilled:

(i) Self-reported doctor-diagnosed stroke or TIA

ICD code consistent with stroke or TIA (I61, I63, I64, G45)
Confirmation by review of clinical notes

Presence of any symptomatic macrovascular disease ('any MVD'; a binary variable) was indicated by a baseline history of TIA, stroke, MI, baseline presence of angina and/or baseline presence of intermittent claudication. Levels of NT-proBNP as well as cIMT and ABI measurements are all indicators of subclinical macrovascular disease, although the term 'subclinical' does not necessarily imply an absence of symptoms. Rather, it is used as a continuous quantification of the relative severity of damage to the vascular system, which may or may not have reached the level of clinical expression.

4.5.3 Use of cognitive variables

Calculation of sum scores

Cognitive data from baseline and the four-year follow-up will be used. Due to relatively strong correlations between the immediate and delayed components of the Faces and the Logical Memory tests respectively (Table 4.4), these were summed for each test and at each time point. Throughout this thesis, the labels 'Faces' and 'Logical Memory' will be used to refer to these sum scores.

Table 4.4: Bivariate correlations of immediate and delayed components of Faces and Logical Memory tests

	Baseline	Year 4
Faces (immediate) and Faces (delayed)	0.55	0.58
LM (immediate) and LM (delayed)	0.87	0.85

Values are Pearson correlation coefficients.
LM, Logical Memory. All $p<0.001$; $n=1050$ (LM) and 1059 (Faces) at baseline; $n=820$ (LM) and 822 (Faces) at year 4.

Imputation of missing cognitive data

As is common in the literature on cognitive ageing (J. F. Price, Stewart, et al., 2008), missing data on the seven cognitive tests of fluid-type ability which were administered at both baseline and at year 4 (LM, Faces, LNS, MR, DSC, TMT-B, BVFT) were imputed. Specifically, accounting for age and sex, missing cognitive test scores were imputed for any participant with missing data on one, two or three out of these seven cognitive tests. This procedure was performed separately for cognitive test scores at baseline and at year 4 follow-up. For participants with missing data on Faces or Logical Memory, the sum scores on the respective test were imputed. A potential for an underestimation of error following imputation may be problematic (Nair, et al., 2007), but in the present analyses was seen to be outweighed by the increased statistical power particularly in the analysis of the global ability factor *g*. The calculation of the factor, which is further described below, requires that data is available on each contributing test, so that any missing data on individual tests would severely restrict the number with participants with a value of *g*. The number of cases with data was imputed is shown separately for each cognitive test in Table 4.5.

Table 4.5: Imputation of cognitive test data at baseline and at year 4

	Baseline		Year 4	
Cognitive test	**Number of cases imputed**	**Data missing following imputation (%)**	**Number of cases imputed**	**Data missing following imputation[a] (%)**
Matrix Reasoning	9	0.28	1	0.72
Digit Symbol Coding	4	0.47	21	0.96
Trail-Making	9	0.47	21	0.96
Logical Memory	12	0.38	5	0.72
Faces	2	0.47	3	0.72
Letter-Number	13	0.47	32	0.84
Verbal Fluency	2	0.38	4	0.60

Log-transformed values were used for Trail-Making. Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test, Letter-Number, Letter-Number Sequencing. [a]total includes three subjects who did not perform any of the cognitive tests at year 4.

Latent variables

A latent variable is a theoretical concept which is not directly accessible to the researcher. It is inferred on the basis of several observed, or manifest, variables assumed to all reflect one underlying construct (Penke & Deary, 2010). For instance, a person's personality may be inferred on the basis of their actions (Borsboom, Mellenbergh, & van Heerden, 2003). Latent variables were first used by Spearman

(1904) who determined the general ability factor *g* on the basis of several individual cognitive tests, but in theory may be applied to any set of correlating variables. . Weighted sumscores of the extracted latent variable constitute an 'operational latent variable', which enables its visualisation as well as its use in further analyses (Borsboom, et al., 2003). Because the variance of the latent variable is shared by all contributing observed variables, latent variables are immune to measurement error, and interpretations of analyses based on latent variables may have higher reliability compared with analyses of observed variables (Penke & Deary, 2010). Following extraction of the latent variable, only test-specific and error variance remains in the individual manifest variables, and correlations amongst these variables is reduced.

Principal component analysis (PCA) and common factor analysis are methods to derive principal components and latent factors respectively. Both reduce the number of variables, but whereas common factor analysis has a confirmatory approach, PCA may be either confirmatory or exploratory. Factor analysis further assumes that observed variables have a causal influence on the latent variable; latent variables derived from PCA are assumed to exert causal influence on the observed variables (Albright & Park, 2009).

The present analyses applied PCA both on cognitive data (to derive the global ability factor *g*) and on risk factor data to derive an 'inflammation' factor. As is common in the literature (Widaman, 1993), components with Eigenvalues>1 will be extracted. Standardised regression scores were saved and used as 'operational latent variables' in further analyses of the respective latent construct.

General ability factor 'g'

In order to derive a construct similar to Spearman's *g* (1904), a PCA was applied to the imputed data on the seven fluid-type neuropsychological tests (LNS, BVFT, MR, DSC, TMT-B, Faces, LM) which are all assumed to reflect general cognitive ability to some extent. Log-transformed values were used for any cognitive test originally identified to be non-normally distributed (described below). The MHVS was not included in the PCA, because its scores are assumed to reflect a crystallised-type ability, which is largely immune to age-related declines, rather than current fluid-type ability, which is the main outcome of interest in the present thesis. The MMSE was also excluded due to its role as a screening instrument for dementia. Baseline *g* and year 4 *g* must reflect the same underlying construct in order to allow investigate change in *g* between the two time points. Therefore, the Deary-Liewald reaction time task was omitted from the PCA on year 4 data.

At baseline, the internal consistency amongst the seven cognitive tests (following imputation of missing data) was found to be acceptable (Cronbach's $\alpha = 0.63$), and so a PCA was performed. All seven of the imputed cognitive tests loaded on a single component, with loadings ranging from 0.47 (Faces) to 0.80 (-TMT-B) (Table 4.6). The derived *g* factor explained 44.74% of total variance. A standardised regression score of the factor was saved, creating a value of baseline *g* for each participant, which was based on imputed cognitive test data.

Table 4.6: Factor loadings of baseline cognitive tests on *g*

Cognitive test	Factor loadings
-Trail-Making	0.80
Digit Symbol Coding	0.75
Letter-Number Sequencing	0.72
Matrix Reasoning	0.67
Verbal Fluency	0.67
Logical Memory	0.54
Faces	0.47

N=1060. The log-transformed variable was used for Trail-Making. Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency test. Data from each cognitive test were imputed prior to extraction of *g*.

The procedure was repeated on the year 4 follow-up cognitive tests (following imputation of missing data) in order to also obtain one value of follow-up *g* for each 'attender' of the follow-up clinic. Initially, internal consistency of the seven year 4 cognitive tests was assessed and found to be acceptable (Cronbach's $\alpha = 0.66$), before all seven cognitive tests were entered into a PCA and again a single component was identified which captured performance on all seven cognitive tests. Factor loadings ranged between 0.51 (Faces) and 0.81 (-TMT-B) (Table 4.7). The component explained 47.44% of total variance in follow-up cognitive test performance.

Table 4.7: Factor loadings of follow-up cognitive tests on *g*

Cognitive test	Factor loadings
-Trail-Making	0.81
Digit Symbol Coding	0.79
Letter-Number Sequencing	0.75
Matrix Reasoning	0.69
Verbal Fluency	0.62
Logical Memory	0.61
Faces	0.51

N=823. The log-transformed variable was used for Trail-Making. Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency test. Data from each cognitive test were imputed prior to the extraction of *g*.

Because the decision to impute missing cognitive data was not made immediately at the beginning of this project, two PCA were initially performed to extract baseline *g* and follow-up *g* on the basis of original data, i.e. without imputation of missing values. These were used for analyses reported in the first publication made on the basis of this thesis (Feinkohl et al., 2012). The overall differences in cognitive test variables (original versus imputed data) used in this thesis and in publications are summarised in Table 4.8. Note that because little cognitive data was imputed for individual cognitive tests (Table 4.5), results at least for analyses of individual cognitive tests are close to identical when using imputed and when using original data throughout.

Table 4.8: Cognitive data in present thesis and in publications

	Seven cognitive tests used to extract *g*	***G***
Feinkohl et al. 2012	Original data (non-imputed)	Original data (non-imputed)
Thesis	Original data (non-imputed)	Imputed
Feinkohl et al., 2013	Imputed	Imputed
Feinkohl et al., 2014	Imputed	Imputed

A comparison of the number of subjects with data available on *g* when this was extracted from imputed cognitive data and when it was extracted from original, non-imputed cognitive data shows that the imputation of missing data led to a substantial increase in statistical power (Table 4.9). This was particularly the case for the analyses of four-year change in *g*, which requires data on both baseline *g* and follow-up *g*. Here, data from 91 participants (11.1% of 'attenders') would have been 'lost', had analyses been restricted to original data.

Table 4.9: Number of participants with data on *g* prior to and following imputation of scores on individual cognitive tests then used to calculate *g*

	N based on original non-imputed cognitive data	N based on imputed cognitive data
Baseline *g*[a]	1021	1060
Follow-up *g*[b]	758	823
Four-year change in *g*[b]	731	822

[a]for all baseline participants (total n=1066)
[b]for attenders of year 4 follow-up (total n=831)

Latent inflammation factor

Because all four inflammatory markers (c-reactive protein, CRP; interleukin-6, IL-6; tumor necrosis factor-alpha, TNF-α; fibrinogen) measured at baseline are expected to be highly correlated, it was anticipated that a PCA of these observed variables would extract components which reflect the shared variance amongst the inflammatory markers. As is common practice in the literature, the first unrotated component from this analysis was used in further analyses. All four inflammatory markers loaded on a single principal component (Table 4.10), which explained 49.68% of variance. The standardised regression score was saved and used as an 'inflammation factor' in further analyses, although the label 'factor' is not strictly correct. In support of the application of a PCA to the four inflammatory markers, the internal consistency of the scale of the markers was found to be acceptable (Cronbach's $\alpha = 0.63$).

Table 4.10: Factor loadings of 'inflammation factor'

CRP	0.81
Fibrinogen	0.76
IL-6	0.76
TNF-α	0.44

N=1038. Log-transformed values were used for CRP, IL-6 and TNF-α.
CRP, c-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor α.

4.5.4 Statistical Analyses

Description of data

Means and standard deviations are reported for continuous cognitive and risk factor variables. Histograms of distributions are visually examined for non-normality; variables with skewed distributions were selected for transformation to their natural logarithmic values or square roots. Instead of means, geometric means (means calculated on the log-transformed scale which are then back-transformed to the original scale of measurement) are reported for these variables in the description of data. Categorical variables are described in terms of frequency of their levels. Patterns of missing data (prior to imputation) are summarised for cognitive data from baseline and year 4 and for baseline risk factor data.

Cut-point for statistical significance

As is common practice in the literature, the statistical cut-point $p<0.05$ is used initially to reject the null-hypothesis, reflecting a <5% probability that the null-hypothesis is rejected despite being true. However, the risk of this type of error (Type I error) increases with increasing number of individual analyses. Analyses may be adjusted for multiple comparisons in order to account for this type of error. In a Bonferroni correction, the level of significance sufficient to reject the null-hypothesis is divided by the number of the individual analyses. For instance, 100 individual analyses requires $p<0.0005$ to reject the null hypothesis. This method is commonly used in exploratory post-hoc analyses, but not when a priori hypotheses are tested (Perneger, 1998). Moreover, Bonferroni correction increases the risk of Type II error- the rejection of a false null-hypothesis. As Perneger (1998) notes, if a strict Bonferroni correction was applied to all analyses, the level at which to halt would be arbitrary. Researchers could apply correction to analyses in a single paper, to a single study or to their entire scientific career. Due to these issues, and because a priori hypotheses which are not independent from one another are tested, the results presented in this thesis are not corrected for multiple comparisons. Instead, the total number of individual analyses described in the main text is kept to a reasonable minimum. Caution is exercised in the interpretation of results with significance marginally below the 5% level; significance levels marginally above this cut-point are interpreted strictly as non-significant.

Outcomes

The overall temporal pattern of the cognitive outcome variables is illustrated in Figure 4.5. The change in cognitive function between baseline and year 4 serves as a 'snapshot' of trajectories of late-life cognitive change of the sample. Its relationship to risk factors measured at baseline is the key interest in this thesis.

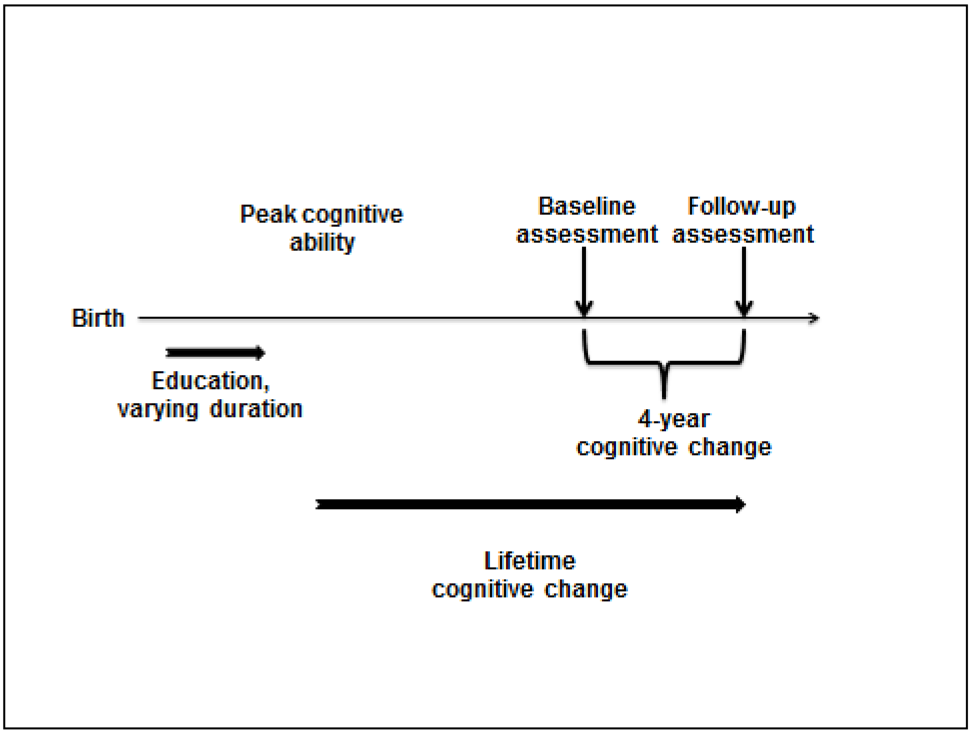

Figure 4.5: Temporal order of cognitive outcome in the ET2DS. Note that in contrast to baseline and follow-up assessments, peak pre-morbid ability was not assessed (estimated) at a fixed time point. Adapted from Glymour, Weuve, Berkman, Kawachi and Robins (2005)

Estimated pre-morbid ability

Baseline scores on the vocabulary-based MHVS are used as estimates of participants' pre-morbid cognitive ability, which is likely to have peaked some time in young adulthood following the completion of education.

Level of cognitive ability

Cognitive test performance and g at year 4 represent the outcome in analyses of cross-sectional relationships between late-life risk factors and late-life level of cognitive function.

Estimated lifetime cognitive change

Adjustment of follow-up cognitive test scores and g for baseline MHVS scores enables the estimation of change in cognitive ability over the course of the lifetime, between peak pre-morbid ability and late-life ability. Results of analyses which use this type of method have previously been found to correlate with actual measures of lifetime cognitive change (Deary, et al., 2004).

Four-year cognitive change

A number of different approaches may be taken to the analysis of longitudinal change in cognitive ability. Consensus on the preferred method is lacked (C. A. Reynolds, Gatz, & Pedersen, 2002), which is important when considering that different approaches may produce conflicting results (Gow, et al., 2008). As a prerequisite to the measurement of change in cognitive function, the cognitive tests must measure identical constructs at both time points (Rogosa, Brandt, & Zimowski, 1982), which necessitated the exclusion of the Deary-Liewald reaction time task from the calculation of year 4 g.

One option for the identification of individuals with decline in cognitive test scores is the application of subjective or objective cut-off points. These may be made on the basis of the distribution of scores in the population (e.g., change from top quartile at baseline to bottom quartile of the distribution at follow-up), or when individuals score at least one standard deviation or standard error of measurement (SEMEAS) below their baseline scores at follow-up. The latter method may be advantageous because it is able to account for practice effects (C. A. Reynolds, et al., 2002). However, categorisation of participants into those who decline in cognitive ability and those who do not results in a loss of statistical power, and may be unable to capture the complexity of patterns linking risk factors with cognitive decline.

Analyses of change measured on the basis of continuous variables avoid such issues. In one approach, 'change scores', or 'gain scores', between two waves of a study are calculated for each participant. These reflect pre- to post-exposure change or change between two randomly chosen time points. 'Annual rate of change' scores (ARC) are a type of change scores based on the ratio of observed change and the number of years over which the change occurred (C. A. Reynolds, et al., 2002). Due to their simplicity, calculation of change scores were initially appealing in psychological research, but lost popularity in the 1960s and 1970s (Willett, 1994).

The approach is flawed because change is continuous, and when based on two randomly chosen time points change scores are essentially only 'snapshots' of change. Problems arise when change correlates with initial status, meaning that individuals with high initial status may have high gains (or low declines) between the two waves (Willett, 1997). Willett (1997, p. 216) describes such correlations as "an almost inevitable fact of life", which may apply to cognitive ageing research in particular because some evidence suggests that cognitive reserve may influence the rate of late-life decline (Gow, et al., 2012). Furthermore, when change occurs at similar rates for all individuals of the sample, reliability of change scores is low (Willett, 1997). This is reflected in the observation that cognitive ARC are typically zero or even positive in samples of cognitively unimpaired older adults (C. A. Reynolds, et al., 2002). Change scores are also affected by regression to the mean (RTM). At each point of observation, random error distorts the true mean of the sample. Values then approach the true value during repeated measurement. Thus, following relatively high or low cognitive test scores at time one, it is likely that a participant performs closer to the true mean at time two (Barnett, van der Pols, & Dobson, 2005; Krum & Tonkin, 2003), so that the change scores then do not reflect the actual cognitive change at all. The use of change scores is further complicated by the observation that RTM is more likely to cause high-ability individuals to perform poorly than it is to cause low-ability individuals to perform better than expected (Gow, et al., 2012). A commonly cited real-life example of RTM is the observation that tall parents tend to have shorter children and vice versa. Repeated measurements of blood pressure, cholesterol or body weight also regress to the mean (Bland & Altman, 1994); 'regression to the truth' describes the observation that initially successful drug trials typically become unsuccessful in later trials (Bland & Altman, 1994; Krum & Tonkin, 2003).

Yet, change scores are unbiased, and reliability may be high at least when individual differences in true change is large (Rogosa & Willett, 1983). Reliable change indices (RCI), which are effect sizes based on the ratio of observed change and the standard error of the change, may also avoid risk of RTM and measurement error (Stein, Luppa, Brähler, König, & Riedel-Heller, 2010). Due to these advantages, it has been suggested that "the difference score is not the outcast that many critics have claimed" (Willett, 1994, p. 673).

Adjustment of follow-up scores for baseline scores ('adjustment method') rivals change scores as a continuous representation of change. The approach models change over time under the assumption that each participant started out with equal initial scores (Willett, 1994) and is common in the literature on cognitive ageing (Gow, et al., 2008) as well as other health outcomes (Yanez, Kronmal, & Shemanski, 1998). The adjustment method and change scores typically lead to identical conclusions (Willett, 1994). Due to the aforementioned flaws of change scores, the former method is chosen for the analyses presented in the main text of this thesis. Year 4 cognitive test scores are adjusted for baseline scores on the respective cognitive test. The analysis of change in *g* requires a different approach to the assessment of the individual tests. Because *g* is not an absolute value, it is not possible to adjust follow-up *g* standardised on attenders of year 4 follow-up for baseline *g* standardised on baseline participants. In order to allow application of the adjustment method, both baseline *g* and year 4 *g* must be standardised on the same population. For this purpose, a third separate PCA was carried out which follows a procedure previously described in Gow et al. (2008): (i) cognitive data of all attenders of the year 4 clinic were arranged in the statistical program so that each of seven columns include cognitive scores obtained at baseline and at follow-up, (ii) a single PCA was performed, (iii) saved regression scores of the first principal component were separated into two columns according to time point. This resulted in baseline *g* and follow-up *g* with identical factor loadings, which ranged between 0.43 (Faces) and 0.79 (-TMT-B) (Table 4.11). The factor accounted for 43.41% of variance in the data.

Table 4.11: Factor loadings of baseline and year 4 follow-up cognitive tests on *g* used to calculate 'four-year change in *g*' variable

Cognitive test	Factor loadings
-Trail-Making	0.79
Digit Symbol Coding	0.75
Letter-Number Sequencing	0.72
Matrix Reasoning	0.67
Verbal Fluency	0.65
Logical Memory	0.55
Faces	0.43

The log-transformed variable was used for Trail-Making. Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test. Data from each cognitive test were imputed.

Finally, the standardised residual from a linear regression of follow-up *g* on baseline *g* derived in this method was saved, and is used to represent 'four-year change in *g*' for each attender of the four-year follow-up.

Main analyses

Univariate analyses

In initial univariate analyses, baseline cognitive test performance, demographics and risk factors are compared between participants lost to attrition between baseline and year 4 and attenders of year 4 follow-up, and between participants with suspected dementia and the remaining sample. The various baseline predictor variables are also associated with each other in order to ascertain relationships amongst risk factors. Correlation coefficients are calculated for cognitive tests scores at baseline and year 4, and mean cognitive test scores are compared between baseline and year 4 for attenders. Further analyses determine associations of age, sex, socioeconomic status and duration of diabetes with predictors and cognitive outcome in order to determine their potential use as covariates in multivariable analyses. The univariate statistical methods include Pearson correlations, point bi-serial correlations, t-tests, chi^2 tests and analyses of variance (ANOVAs). All analyses are two-tailed.

Multivariable analyses linear regression analyses

For completeness, all individual risk factors as well as the 'inflammation' factor are then associated with all cognitive outcomes (estimated pre-morbid ability, baseline ability, four-year cognitive change and estimated lifetime cognitive change, with the latter three outcomes represented both in terms of *g* and all seven individual cognitive tests) in linear regression analyses. Results from these analyses are presented in Appendix D. Analyses associating risk factors with four-year cognitive change are additionally selected as an example analysis which are repeated using raw scores of cognitive change instead of the adjustment method (Appendix E). Although all further analyses presented in this thesis on four-year cognitive change employ the adjustment method, a comparison of the findings in Appendix D and Appendix E demonstrate an overall similarity of findings (particularly with respect to effect sizes) following both of these approaches.

In the main part of the multivariable analyses, linear regression analyses and analyses of covariance (ANCOVA) determine associations of baseline subclinical and symptomatic macrovascular disease variables, of baseline history of severe hypoglycaemia (SH), and of incident SH between baseline and year 4 with cognitive outcome (estimated peak pre-morbid ability, baseline ability, four-year cognitive change and estimated lifetime cognitive change). Due to evidence of hypoglycaemia as a consequence of poorer cognitive ability, incident SH between baseline and year 4 is additionally investigated as a potential outcome of poorer baseline cognitive ability in ANCOVAs and in logistic regression analyses is used to calculate odds ratios.

A total of 15 metabolic and vascular risk factor variables measured at baseline/ year 1 are then associated with the level of late-life cognitive function, with estimated lifetime cognitive change and with four-year cognitive change with the aim to compare relative predictive ability with respect to late-life cognitive outcome. For this purpose, stepwise linear regression analyses, which are further described in Chapter 8, sees the addition of all 15 risk factors into models of follow-up *g*, estimated lifetime change in *g* and four-year change in *g*, with retention of those with associations with $p<0.05$ in each step. All analyses are performed in SPSS version 19.0 (IBM Corporation, New York).

Chapter 5: Results I: Characteristics of study population and descriptive statistics for risk factor and cognitive test variables

This chapter describes the characteristics and representativeness of the ET2DS study population, presents descriptive statistics for the risk factor variables included in subsequent analyses and describes the cognitive test results from baseline and follow-up.

5.1 Study population

5.1.1 Baseline socio-demographic characteristics

The participants of the baseline clinic of the ET2DS (n=1066) were on average 67.9 years old, and 547 (51.3%) participants were male. One hundred and twenty-seven (11.9%) were in the first quintile (most deprived), 208 (19.5%) were in the second quintile, 188 (17.6%) were in the third quintile, 194 (18.2%) were in the fourth quintile and 349 (32.7%) were in the top quintile (least deprived) of the Scottish Index of Multiple Deprivation (SIMD). A majority of participants (n=581; 54.6%) self-reported secondary school as their highest educational attainment, 307 (28.8%) obtained other professional or technical qualifications after leaving school, 171 (16.0%) had a University degree, and seven individuals (0.7%) had terminated education following primary school.

5.1.2 Representativeness

Representativeness of the recruited sample (n=1066) as successfully capturing older adults with type 2 diabetes living in the Lothian area was determined by researchers involved in the baseline phase of the study through comparison of clinical and socio-demographic characteristics of the study population recorded on the Lothian Diabetes Register with those of individuals who had been invited to participate but declined or did not respond (‘non-responders’; n=4386) (Marioni, Strachan, et al., 2010). The recruited sample and non-responders were found to be similar in social class (SIMD) ($p>0.05$) and age (67.9 years ± 4.2 versus 67.9 years ± 4.4; $p>0.05$). In the recruited sample, 516 individuals (48.4%) had a diabetes duration of ≤5 years compared with 2135 (48.7%) of non-responders (p>0.05). Mean HbA1c was identical for the two groups (7.4% ± 1.12 versus 7.4% ± 1.36; p>0.05). Of the recruited sample, 185 (17.4%) were treated with insulin, compared with 704 (16.1%) of the non-responders (p>0.05). However, the recruited sample was more likely to be male (n=547, 51.3% versus n=1839, 41.9%; p<0.001), and had higher mean total cholesterol (4.2 mmol/l ± 0.96 versus 4.3 mmol/l ± 0.90; p<0.001) and lower systolic blood pressure (137.2 mmHg ± 18.2 versus 133.3 mmHg ± 16.4; p<0.01) compared with non-responders, although differences were not large (Marioni, Strachan, et al., 2010).

In order to determine the representativeness of the ET2DS sample relative to other older populations with type 2 diabetes, prevalence of disease categories and mean values of continuously assessed clinical measures (Table 5.2) were compared with those reported in previous studies.

Similar to the prevalence of stroke in the ET2DS (5.8%), the Utrecht Diabetic Encephalopathy Study of patients with a similar mean age (mean 66 years) and duration of diabetes (mean 8.7 years) compared with the ET2DS (mean age 68 years; mean duration of diabetes 8.1 years at baseline), had reported a similar prevalence of 6% (Manschot et al., 2007). However, prevalence of MI and/or angina appeared to be lower in this study (31%) compared with the ET2DS (42%).

With 6.1%, the ET2DS had apparently lower prevalence of PAD than reported in previous studies (11%, Manschot et al., 2007; 10% Potier et al., 2007; 29%, Hirsch et al., 2001) although the latter two investigations defined PAD on the basis of ABI which may have affected levels of prevalence reported in the studies. ABI appeared to be somewhat lower in the ET2DS (mean 0.98) than in a Chinese study which was comparable in terms of demographic characteristics (mean 1.12) (Chen et al., 2011).

Very similar to the ET2DS (32.5%), retinopathy was prevalent in 33% of subjects in the Dutch study (Manschot et al., 2007). Mean HbA1c measured at the baseline clinic (7.4%) appeared to be similar to those reported for the Utrecht Diabetic

Encephalopathy Study (6.9%) (Manschot et al., 2007) and those reported for the Anglo-Danish Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care (ADDITION) Study (6.1%) (Ruis et al., 2009). Similar findings were made when comparing BMI in the ET2DS (mean 31.4) with the Utrecht Diabetic Encephalopathy Study (mean 28.1) (Manschot et al., 20078) and with ADDITION (mean 30.4) (Ruis et al., 2009). Relatively large apparent differences in BMI to the Chinese study (mean 23.7) could potentially be due to cultural differences between Scottish and Chinese older adults with type 2 diabetes which may influence their clinical characteristics (Chen et al., 2011)

With 14%, prevalence of current smoking was notably lower in the ET2DS compared with the Utrecht Diabetic Encephalopathy Study (22%) (Manschot et al., 2007), ADDITION (21%) (Ruis et al., 2009) and the Chinese cohort (19%) (Chen et al., 2011). Finally, cholesterol levels and systolic blood pressure appeared to be relatively lower in the ET2DS compared with other samples (e.g., mean HDL cholesterol 4.3 mmol/l in Manschot et al., 2007; mean systolic blood pressure 143 mmgH in ADDITION, Ruis et al., 2009).

Of those subjects who were treated with insulin at year 4, 4.9% reported incident severe hypoglycaemia since baseline, resulting in an annual incidence of around 1.3%. This incidence is relatively lower than that reported by the Hypoglycaemia Study Group for older participants with type 2 diabetes who are on insulin. Here, prevalence was estimated to be around 3-15% depending on the duration of treatment (UK Hypoglycaemia Study Group, 2007). It may be the case that participants of the ET2DS were selected for a shorter duration of insulin treatment, adherence to treatment or for cognitive ability, all of which may have offered protection from severe hypoglycaemia.

Comparisons of the ET2DS sample with other key population-based cohorts with type 2 diabetes were not made due to differences in demographic characteristics, such as age and disease duration (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008; Wu et al., 2003). Comparisons with ADVANCE (de Galan et al., 2009) and ACCORD-MIND (Punthakee et al., 2012) were also neglected due to specific selection criteria having been applied during recruitment to these trials. ADVANCE selected subjects with presence of major macrovascular or microvascular disease disease and/or increased vascular risk, for instance. Consequently, their samples may not be representative of the general older population living with type 2 diabetes.

Overall, the participants of the ET2DS appeared to be relatively representative in terms of lifestyle and clinical characteristics both to the population with type 2 diabetes living in the Lothian area of Scotland (as far as the data on the Lothian Diabetes Register showed), and to other populations-based studies of older adults with type 2 diabetes as reported in the literature.

5.2 Risk factor distributions and inter-correlations

For all risk factors (except severe hypoglycaemia), only data collected at baseline or at year 1 were used in the analyses reported in this thesis and so are described here. This section reports missing risk factor data and distributions, inter-correlations amongst risk factors, and their associations with age, sex and duration of diabetes.

5.2.1 Missing data

As shown in Table 5.1, a majority of risk factor variables had very little missing data (<4%). Data was complete for age and all binary disease variables (TIA, stroke, angina, MI, PAD, dementia), because only individuals with evidence for the respective disease or event were recorded; all remaining individuals constitute the respective ‘no disease/event’ group. However, some may have been included in this group due to missing data despite having suffered the disease or event. This is likely to weaken findings in ‘disease/event’ versus ‘no disease/event’ analyses, but is inherent to the nature of these risk factor categories and their determination.

Table 5.1: Missing data on risk factors measured at baseline or year 1

	N	Missing in % of baseline sample	Reason (if available)
Current anti-diabetic treatment	1065	0.09%	
HADS-A	1065	0.09%	
HADS-D	1065	0.09%	
Body Mass Index	1065	0.09%	
Systolic bp at clinic visit	1064	0.19%	
IL-6	1064	0.19%	
TNF-α	1063	0.28%	
Fibrinogen	1063	0.28%	
Historical systolic bp	1063	0.28%	
Cortisol	1062	0.38%	
Historical HbA1c	1062	0.38%	
Waist-hip ratio	1061	0.47%	
ABI	1059	0.66%	Amputations
LDL:HDL	1057	0.84%	
Duration of diabetes	1053	1.22%	
NT-proBNP	1050	1.50%	Secondary analysis of blood samples
Diabetic retinopathy	1044	2.06%	Retinal photographs taken at separate appointment
CRP	1043	2.16%	
Inflammation 'factor'	1038	2.63%	Required data on all four inflammatory markers
History of severe hypoglycaemia	1034	3.00%	Uncertainty was coded as 'missing'
Alcohol units/year (quintiles)	1032	3.19%	
HbA1c at clinic visit	1028	3.56%	
Packyears	1019	4.41%	
Carotid IMT	917	14.0%[a]	Measured at year 1
6-month survey of severe hypoglycaemia	898	15.8%[b]	Carried out following year 1 clinic; some non-responders

[a]data missing for 2.34% of year 1 sample. [b]data missing for 4.37% of year 1 sample.
HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale- Depression subscale; ABI, ankle brachial index; bp, blood pressure; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, c-reactive protein; LDL:HDL, low-density lipoprotein: high-density lipoprotein ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; carotid IMT, carotid intima-media thickness.

Because a relatively large proportion of data was missing for packyears, cIMT and the 6-month survey of severe hypoglycaemia (SH) (all >4%), individuals with and those without data on these measures were compared in terms of socio-demographic characteristics (age, sex, education, SIMD) and baseline global cognitive ability measured by *g*. Compared with the respective remaining baseline populations, the group with missing data on packyears was more likely to be male (p=0.003) and had lower baseline *g* (mean -0.31 ± 0.85 versus 0.01 ± 1.00; p=0.032). The group with missing cIMT data was of lower social class (SIMD; p=0.030) and had lower baseline *g* (mean -0.44 ± 1.05 versus 0.07 ± 0.97; p<0.001). The group who did not participate in the 6-month survey of SH had lower baseline *g* (mean -0.34 ± 1.09 versus 0.07 ± 0.97; p<0.001). All of the remaining socio-demographic parameters were similar in the groups with missing data and the respective remaining populations (all p>0.05).

It is plausible that the findings for cIMT and 6-month SH directly reflect the attrition of lower-cognitive-ability individuals between baseline and year 1 (at which point cIMT was measured and participants were enrolled in the 6-month survey of SH). Overall, the tendency for lower cognitive ability in individuals with missing data on packyears, cIMT and 6-month SH has the potential to affect the results of analyses of these risk factors and cognitive outcome, and will therefore be considered in the interpretation of the results.

5.2.2 Risk factor distributions

Descriptive statistics for baseline and year 1 risk factors

Mean ± SD, median (quartile range) or n (%) for risk factor variables are presented in Table 5.2. Note that the table reports data on risk factors which are not always used in any subsequent analyses in this thesis, but they describe important general characteristics of the population.

Table 5.2: Baseline and year 1 risk factor data

	Mean ± SD, median (quartile range) or n (%)	Minimum	Maximum
Duration of diabetes (years)	6 (3 – 11)	0	43
Current treatment			
Insulin +/-tablets	186 (17.5)		
Tablets alone	679 (63.8)		
Diet alone	200 (18.8)		
HbA1c at clinic visit (%)	7.4 ± 1.1	5.0	14.9
Average historical HbA1c (%)	7.4 ± 0.9	4.9	11.6
Plasma glucose (mmol/L)	7.2 (6.2 – 8.5)	2.1	22.2
Systolic bp at clinic visit (mmHg)	133.3 ± 16.4	90	210
Diastolic bp at clinic visit (mmHg)	69.1 ± 9.0	20	110
Average historical systolic bp (mmHg)	138.3 ± 10.2	105	185
Macrovascular disease			
Myocardial infarction	150 (14.1)		
Angina	298 (28.0)		
Stroke	62 (5.8)		
TIA	31 (2.9)		
Claudication	65 (6.1)		
Any MVD	393 (36.9)		
DR severity			
None	705 (67.5)		
Mild	292 (28.0)		
Moderate/severe	47 (4.5)		
Carotid IMT (mm)	1.00 ± 0.17	0.63	1.70
Ankle brachial index	0.98 ± 0.21	0.27	2.34
NT-proBNP(pg/ml)	75 (37 – 169)	5	2926
Body Mass Index (kg/m^2)	31.43 ± 5.69	18.40	55.44
Waist-hip ratio	0.97 ± 0.08	0.74	1.22
Total cholesterol (mmol/L)	4.3 ± 0.9	2.3	9.4
HDL cholesterol (mmol/L)	1.3 ± 0.4	0.4	3.3
LDL:HDL ratio	3.5 ± 1.1	1.7	10.7
Smoking			
Current smoker	153 (14.4)		
Ex-smoker	499 (46.8)		
Never smoked	414 (38.8)		
Packyears	7.20 (0.0 – 32.0)	0.0	180.0
Alcohol units/year groups			
0 (abstainers)	298 (27.7)		
1	187 (17.4)		
2	226 (21.0)		
3	172 (16.0)		
4	149 (13.9)		
CRP (mg/l)	1.86 (0.87 – 4.38)	0.10	160.00

Fibrinogen (g/l)	3.65 ± 0.74	0.70	7.14
IL-6 (pg/ml)	2.87 (1.96 – 4.47)	0.49	34.18
TNF-α (pg/ml)	1.07 (0.69 – 1.62)	0.10	28.00
Inflammation 'factor'	0.00 ± 1.00	-2.39	4.67
Cortisol (nmol/l)	722.2 ± 196.3	49.5	1447.8
MMSE	29 (28 – 30)	14	30
MMSE<24	30 (2.8)		
Dementia	19 (1.8)		
HADS-A	5 (3 – 8)	0	20
HADS-D	3 (1 – 6)	0	16

Total n=1066. Carotid IMT and 6-month incident SH measured at year 1.

Any MVD, any symptomatic macrovacular disease; TIA, transient ischaemic attack; HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale- Depression subscale; MMSE, Mini-Mental-State Examination; bp, blood pressure; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, c-reactive protein; LDL:HDL, low-density lipoprotein: high-density lipoprotein ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; DR, diabetic retinopathy; SH, severe hypoglycaemia; carotid IMT, carotid intima-media thickness.

Distributions of continuous baseline and year 1 risk factor variables

Histograms of the continuous risk factor variables were visually inspected (Figure B.1 to B.28 in Appendix B). Positively skewed distributions were identified for NT-proBNP, packyears, alcohol units/year, HADS-D, HADS-A, diabetes duration, plasma glucose, CRP, IL-6 and TNF-alpha; all of the remaining risk factor variables were approximately normally distributed. Prior to their use in further analyses, the variables with skewed distributions (except packyears) were each transformed to their natural logarithms. Because HADS-D, HADS-A, alcohol units/year and disease duration could each have values of 0 (which cannot be log-transformed), 1 was added to each HADS-D, HADS-A, alcohol units/year, and disease duration value prior to transformation. Subsequent log-transformation achieved approximately normal distribution for all variables with previously skewed distributions (Figure B.29 to B.37 in Appendix B). The packyears variable was transformed to its square root, because this led to a distribution which was closer to normal compared with its log-transformation.

Descriptive statistics for severe hypoglycaemia (SH)

Data on SH were used in terms of prevalence of a lifetime history at baseline (self-reported at baseline), incidence between baseline and year 4 (self-reported at year 4), and incidence within the 6-month survey carried out immediately after the year 1 clinic. The reporting of SH over the course of the study is shown in Figure 5.1. At baseline, 112 participants (10.5% of baseline population) reported a lifetime history of SH. A majority of these individuals (n=57; 50.9%) had experienced 1 or 2 episodes, 28 (25.0%) reported 3 to 4 episodes, and 27 (24.1%) had experienced 5 or more episodes. Of the 112 participants with a baseline history of SH, 51 (45.5%) were on insulin treatment, 53 (47.3%) were on tablets, and 8 (7.13%) were treated by diet alone at the time of the baseline assessment.

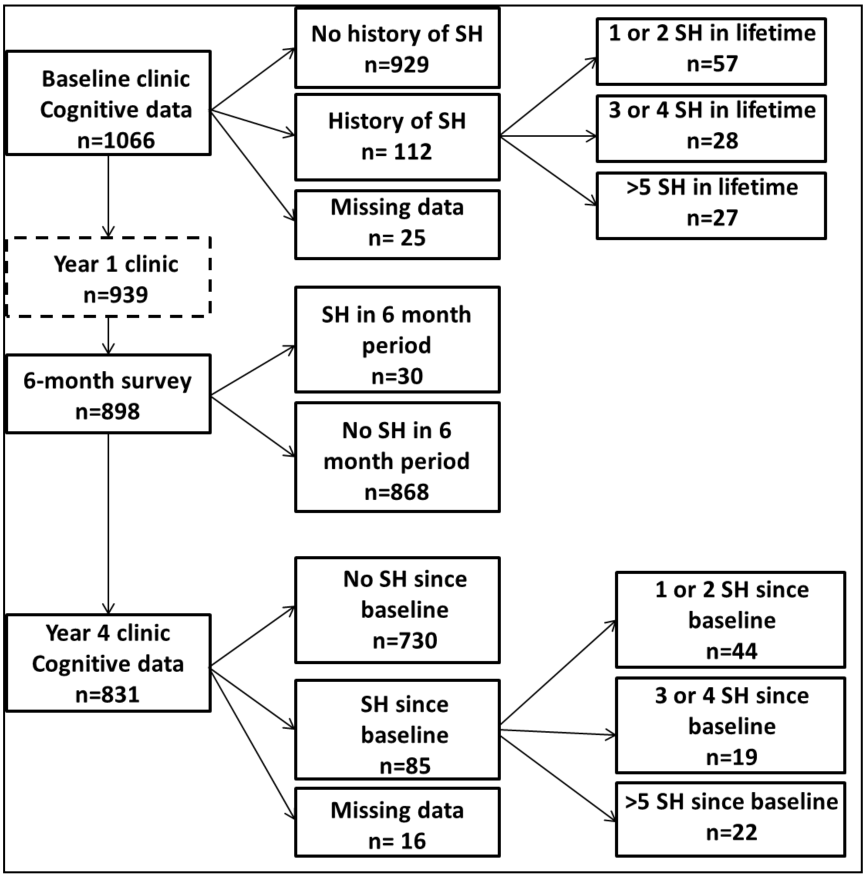

Figure 5.1: Severe hypoglycaemia in the ET2DS

At the year 4 clinic, 85 participants (10.2% of the year 4 population) reported incident SH between baseline and year 4 follow-up (Figure 5.1). Of these, most (n=44; 51.8%) again had been affected by 1 or 2 episodes since the baseline clinic visit, 19 (22.4%) had experienced 3 to 4 episodes and 22 (25.9%) suffered 5 or more episodes. Of the 85 participants with incident SH, 40 (47.1%) were on insulin treatment at the time of the year 4 clinic, 41 (48.2%) were on tablets alone, and 4 (4.7%) were treated by diet. Twenty-seven (31.8%) of the 85 participants with incident SH had also been in the group with a lifetime history of SH reported at baseline. In the 6-month survey of SH, 30 participants (3.3% of individuals enrolled in the survey) reported a total of 45 episodes. For 18 of these, blood glucose readings were self-reported. All but one of 14 measurements made prior to treatment were below 3.0mmol/l. Of the 30 participants reporting SH in the 6-month survey, 25 recovered following treatment with sugary drink or food, one required injection with glucagon and three required intravenous glucose (data missing for one subject). Twenty-three of the 30 subjects attended the year 4 follow-up.

5.2.3 Associations of risk factors with age, sex and disease duration

To summarise the statistically significant associations of risk factors with age, sex and disease duration (Table 5.3), age correlated positively with disease duration, blood pressure, HDL cholesterol, cIMT, NT-proBNP and HbA1c, and with all inflammatory markers except CRP, as well as with the derived inflammation ‘factor’, and correlated negatively with alcohol consumption, total cholesterol and LDL:HDL. Longer disease duration predicted higher inflammatory marker levels (except CRP) and higher values on inflammation ‘factor’, and was further associated with higher waist-hip ratio, higher NT-proBNP and higher HbA1c. Females tended to have higher HbA1c, lower waist-hip ratio, LDL:HDL, ABI and cIMT and had fewer packyears and lower yearly alcohol consumption compared with males. Males had lower levels of fibrinogen and CRP, and lower overall inflammation.

As shown in Table 5.4, individuals with any symptomatic macrovascular disease, myocardial infarction and angina were older and had longer disease duration compared with the respective remaining populations. Additionally, patients with a history of stroke, those with symptomatic PAD and those with diabetic retinopathy tended to have longer disease duration. Males were more likely to suffer from any symptomatic macrovascular disease and angina and to have suffered myocardial infarction or stroke.

Table 5.3: Age, sex and disease duration associations with continuous baseline and year 1 risk factors

	Age	Duration of diabetes	Sex		
			Males	**Females**	**p-value**
Duration of diabetes (years)	**0.07 (0.018)**	--	6 (4 – 11)	6 (3 – 10)	0.16
Historical HbA1c (%)	**-0.07 (0.023)**	**0.28 (<0.001)**	**7.34 ±0.86**	**7.47 ±0.93**	**0.019**
HbA1c at clinic visit (%)	**-0.06 (0.044)**	**0.28 (<0.001)**	7.36 ± 1.13	7.43 ± 1.10	0.31
Waist-hip ratio	**-0.05 (0.11)**	**0.12 (<0.001)**	**1.00 ± 0.06**	**0.92 ± 0.07**	**<0.001**
Total cholesterol (mmol/L)	**-0.08 (0.013)**	**-0.12 (<0.001)**	**4.15 ± 0.84**	**4.48 ± 0.94**	**<0.001**
HDL cholesterol (mmol/L)	**0.06 (0.050)**	-0.03 (0.29)	**1.21 ± 0.35**	**1.38 ± 0.35**	**<0.001**
LDL:HDL	**-0.11 (<0.001)**	**-0.05 (0.11)**	**3.65 ±1.09**	**3.42 ± 1.06**	**<0.001**
Historical systolic bp (mmHg)	0.05 (0.10)	0.03 (0.37)	137.8 ± 10.0	138.9 ± 10.4	0.09
Systolic bp at clinic visit (mmHg)	**0.08 (0.013)**	-0.01 (0.79)	133.8 ± 15.9	132.8 ± 17.0	0.35
Diastolic bp at clinic visit (mmHg)	**-0.13 (<0.001)**	**-0.11 (0.001)**	**70.5 ± 8.8**	**67.5 ± 9.0**	**<0.001**
Packyears	-0.04 (0.21)	0.00 (0.96)	**10.45 ± 5.42**	**4.05 ± 5.16**	**<0.001**
Alcohol (units/ year)	-0.07 (0.031)	-0.06 (0.08)	**111.93 ± 14.25**	**15.11 ± 12.63**	**<0.001**
Cortisol (nmol/l)	0.03 (0.34)	0.02 (0.46)	726.3 ± 183.4	717.9 ± 209.3	0.49
ABI	-0.02 (0.60)	-0.03 (0.32)	**1.01 ± 0.24**	**0.95± 0.16**	**<0.001**
Carotid IMT (mm)	**0.15 (<0.001)**	0.09 (0.72)	**1.03 ± 0.17**	**0.96 ± 0.16**	**<0.001**
NT-proBNP (pg/ml)	**0.25 (<0.001)**	**0.15 (<0.001)**	68 (35 – 175)	87 (42 – 166)	0.41
TNF-α (pg/ml)	**0.10 (0.002)**	**0.15 (<0.001)**	1.0 (0.7 – 1.6)	1.1 (0.8 – 1.6)	0.13
Fibrinogen (g/l)	**0.07 (0.022)**	**0.11 (0.001)**	**3.5 ± 0.8**	**3.8 ± 0.7**	**<0.001**
CRP (mg/l)	-0.02 (0.52)	-0.04 (0.25)	**1.4 (0.7 – 3.5)**	**2.4 (1.2 – 5.6)**	**<0.001**
IL-6 (pg/ml)	**0.09 (0.003)**	**0.13 (<0.001)**	2.8 (1.9 – 4.4)	2.9 (1.9 – 4.5)	0.52
Inflammation 'factor'	**0.08 (0.016)**	**0.11 (0.001)**	**-0.17 ± 0.95**	**0.18 ± 1.01**	**<0.001**

Analyses are two-tailed Pearson correlations and t-tests. Values for age and disease duration are correlation coefficients (p-values); for sex are means ± SD or median (interquartile range). NT-proBNP, TNF-α, CRP, IL-6. Square root transformed values were used for packyears. Bp, blood pressure; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, c-reactive protein; LDL:HDL, low-density lipoprotein: high-density lipoprotein ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; carotid IMT, carotid intima-media thickness.
Log-transformed or square root transformed variables were used for duration of diabetes, packyears, alcohol, NT-proBNP, TNF-α, CRP, IL-6.

Table 5.4: Age, sex and disease duration associations with categorical baseline risk factors

	Age (years)			Duration of diabetes (years)			Sex in disease group		
	Mean ± SD in disease group	**Mean ± SD in 'no disease'**	**p-value**	**Median (interquartile range) in disease group**	**Median (interquartile range) in 'no disease'**	**p-value**	**N male (%)**	**N female (%)**	**p-value**
Any MVD	**68.45 ± 4.33**	**67.60 ± 4.09**	**0.001**	**7 (4-11)**	**6 (3-11)**	**0.003**	**242 (44.2)**	**151 (29.1)**	**<0.001**
Stroke	68.72 ± 3.84	67.86 ± 4.22	0.12	**8 (6 – 16)**	**6 (3 – 11)**	**0.001**	**43 (7.9)**	**19 (3.7)**	**0.003**
Transient ischaemic attack	67.97 ± 4.68	67.91 ± 4.19	0.94	7 (4 – 11)	6 (3 – 11)	0.89	19 (3.5)	12 (2.3)	0.26
Myocardial infarction	**68.87 ± 4.13**	**67.76 ± 4.20**	**0.003**	7 (4 – 12)	6 (3 – 11)	0.051	**115 (21.0)**	**35 (6.7)**	**<0.001**
Angina	**68.67 ± 4.23**	**67.20 ± 4.08**	**<0.001**	**7 (4 – 11)**	**6 (3 – 11)**	**0.021**	**182 (33.3)**	**116 (22.4)**	**<0.001**
Peripheral arterial disease	68.24 ± 4.41	67.89 ± 4.19	0.52	**8 (4 – 14)**	**6 (3 – 11)**	**0.027**	39 (7.1)	26 (5.0)	0.15
Diabetic retinopathy	67.94 ± 4.24	67.90 ± 4.18	0.87	**10 (5 – 15)**	**5 (3 – 9)**	**<0.001**	188 (18.0)	151 (14.5)	0.06

Analyses are two-tailed t-tests and chi^2 tests. Values are means ± SD or medians (interquartile range). Disease groups are compared to all other participants. 'Any MVD', any symptomatic macrovascular disease. Log-transformed values were used for t-tests comparing duration of diabetes between disease groups. Diabetic retinopathy is a binary variable.

5.3 Characteristics of follow-up population and prevalence of dementia

The risk factor and demographic characteristics of the year 4 follow-up population ('attenders') versus those lost to attrition between baseline and year 4 ('non-attenders') and the demographic characteristics of individuals with dementia at year 4, are described here.

5.3.1 Attrition between baseline and year 4

Of the total study population (n=1066), 831 returned for the year 4 follow-up (retention rate 78.0%). An overview of the reasons for attrition is presented in Figure 4.1 of the Method chapter. Two-tailed t-tests and chi^2 tests revealed that non-attenders were overall older and were of lower social class compared with attenders (Table 5.5).

Table 5.5: Baseline demographic characteristics of 'attenders' and 'non-attenders' of the year 4 follow-up

	Attenders (total n=831)	Non-attenders (total n=235)	p-value for difference or trend
Age (years)	**67.7 ± 4.2**	**68.7 ± 4.3**	**0.001**
Male sex	430 (51.7)	117 (49.8)	0.60
Education			0.11
University degree	145 (17.4)	26 (11.1)	
Professional qualification	239 (28.8)	68 (28.9)	
Secondary school	442 (53.2)	139 (59.1)	
Primary school	5 (0.6)	2 (0.9)	
SIMD rank			**<0.001**
1st quintile	**99 (11.9)**	**28 (11.9)**	
2nd quintile	**143 (17.2)**	**65 (27.7)**	
3rd quintile	**143 (17.2)**	**45 (19.1)**	
4th quintile	**146 (17.6)**	**48 (20.4)**	
5th quintile	**300 (36.1)**	**49 (20.9)**	

Values are mean ± SD or n (%). SIMD, Scottish Index of Multiple Deprivation

5.3.2 Risk factors in attenders and non-attenders

Participants who returned for year 4 follow-up also tended to have lower blood pressure, lower levels of inflammation, lower NT-proBNP and lower packyears and alcohol consumption, had lower prevalence of symptomatic macrovascular disease and experienced fewer symptoms of anxiety and depression at baseline compared with non-attenders (Table 5.6). Attenders and non-attenders were similar on all of the remaining risk factor parameters.

Table 5.6: Baseline risk factors in attenders and non-attenders of year 4 follow-up

	Attenders		Non-attenders		
	Total N	**Mean ± SD, median (quartile range) or n (%)**	**Total N**	**Mean ± SD, median (quartile range) or n (%)**	**p-value for difference or trend**
Duration of diabetes (years)	824	6 (3 – 11)	229	7 (3 – 12)	0.39
Current treatment					0.28
Insulin +/-tablets	830	139 (16.7)	235	47 (20.0)	
Tablets alone	830	526 (63.4)	235	153 (64.8)	
Diet alone	830	165 (19.9)	235	35 (14.9)	
Historical HbA1c (%)	825	7.39 ± 0.90	230	7.45 ± 0.87	0.35
HbA1c at clinic visit (%)	804	7.39 ± 1.13	224	7.41 ± 1.08	0.87
Severe hypoglycaemia	**816**	**77 (9.4)**	**225**	**35 (15.6)**	**0.009**
Historical Systolic bp (mmHg)	825	138.2 ± 10.0	231	138.9 ± 10.8	0.35
Systolic bp at clinic visit (mmHg)	**829**	**132.5 ± 15.9**	**235**	**136.1 ± 18.1**	**0.003**
Diastolic bp at clinic visit (mmHg)	829	69.0 ± 8.9	235	69.4 ± 9.5	0.46
Macrovascular disease					
MI	831	111 (13.4)	235	39 (16.6)	0.21
Angina	831	222 (26.7)	235	76 (32.3)	0.09
Stroke	831	44 (5.3)	235	18 (7.7)	0.17
TIA	831	27 (3.2)	235	4 (1.7)	0.21
Claudication	831	53 (6.4)	235	12 (5.1)	0.47
Any MVD	**831**	**293 (35.3)**	**235**	**100 (42.2)**	**0.041**
NT-proBNP (pg/ml)	**820**	**71 (35 – 158)**	**230**	**102 (48 – 216)**	**0.004**
Carotid IMT (mm)	775	1.00 ± 0.17	142	0.99 ± 0.17	0.41
ABI	**824**	**0.99 ± 0.20**	**235**	**0.95 ± 0.23**	**0.009**
Waist-hip ratio	828	0.97 ± 0.08	233	0.97 ± 0.08	0.97
Total cholesterol (mmol/L)	826	4.34 ± 0.90	231	4.23 ± 0.91	0.10
HDL cholesterol (mmol/L)	826	1.29 ± 0.36	231	1.31 ± 0.38	0.52
LDL:HDL ratio	826	3.57 ± 1.11	231	2.09 ± 0.98	0.06

Packyears	**803**	**6 (0 – 30)**	**231**	**15 (0 – 40)**	**0.019**
Smoking **Current smoker** **Ex-smoker** **Never smoked**	**831**	 **108 (13.0)** **390 (46.9)** **333 (40.1)**	**235**	 **45 (19.1)** **109 (46.4)** **81 (34.5)**	**0.042**
Alcohol (units/year) groups **0 (abstainers)** **1** **2** **3** **4**	**807**	 **212 (25.5)** **148 (17.8)** **186 (22.4)** **136 (16.4)** **125 (15.0)**	**225**	 **86 (36.6)** **39 (16.6)** **40 (17.0)** **36 (15.3)** **24 (10.2)**	**0.002**
CRP (mg/l)	**817**	**1.7 (0.83 – 3.89)**	**226**	**2.75 (1.10 – 6.77)**	**0.002**
Fibrinogen (g/l)	**829**	**3.61 ± 0.71**	**234**	**3.78 ± 0.83**	**0.003**
IL-6 (pg/ml)	**829**	**2.72 (1.87 – 4.29)**	**235**	**3.40 (2.36 – 5.39)**	**0.002**
TNF-α (pg/ml)	828	1.05 (0.68 – 1.61)	235	1.17 (0.78 – 1.68)	0.24
Inflammation 'factor'	**813**	**-0.08 ± 0.97**	**225**	**0.29 ± 1.07**	**<0.001**
DR severity					0.77
None	819	553 (67.5)	225	152 (67.6)	
Mild	819	231 (28.2)	225	61 (27.1)	
Moderate/ severe	819	35 (4.3)	225	12 (5.3)	
Cortisol (nmol/l)	829	727.1 ± 203.7	233	720.8 ± 194.3	0.67
MMSE	**830**	**29 (28 – 30)**	**233**	**28 (27 – 29)**	**<0.001**
MMSE<24	**830**	**13 (1.6)**	**233**	**17 (7.3)**	**<0.001**
HADS-A	**831**	**5 (3 – 8)**	**234**	**6 (4 – 9)**	**0.007**
HADS-D	**831**	**3 (1 – 5)**	**234**	**4 (2 – 6)**	**0.004**

Values are means ± SD, median (interquartile range) or n (%). Any MVD, any symptomatic macrovascular disease; MI, myocardial infarction; TIA, transient ischaemic attack; HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale- Depression subscale; MMSE, Mini-Mental-State Examination; bp, blood pressure; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, c-reactive protein; LDL:HDL, low-density lipoprotein: high-density lipoprotein ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; DR, diabetic retinopathy; carotid IMT, carotid intima-media thickness. Log-transformed or square root transformed values were used for analyses of duration of diabetes, NT-proBNP, packyears, CRP, IL-6, TNF-α, HADS-A, HADS-D and MMSE.

5.3.3 Prevalence of dementia

Although the ET2DS aimed to recruit cognitively healthy older individuals, 19 subjects met the criteria for dementia specified in Section 4.4.5 at the end of the four-year follow-up (2011). Of these, only four had attended the year 4 clinic; 15 had been lost to attrition following baseline. The dementia group was overall older and of lower education compared with the remaining baseline population, but was of a similar social class (Table 5.7). For full baseline risk factor characteristics in the dementia group versus subjects without dementia diagnosis, see Appendix A.

Table 5.7: Demographic characteristics of dementia patients and the remaining baseline sample

	Dementia (total n=19)	Remaining sample (total n=1047)	p-value for difference or trend
Age	**71.1 ± 3.4**	**67.9 ± 4.2**	**0.001**
Male sex	11 (57.9)	538 (51.2)	0.56
Education			**<0.001**
University degree	**3 (15.8)**	**168 (16.0)**	
Professional qualification	**4 (21.1)**	**303 (28.9)**	
Secondary school	**10 (52.6)**	**571 (54.5)**	
Primary school	**2 (10.5)**	**5 (0.5)**	
SIMD rank			0.43
1st quintile	4 (21.1)	123 (11.7)	
2nd quintile	5 (26.3)	203 (19.4)	
3rd quintile	1 (5.3)	187 (17.9)	
4th quintile	4 (21.1)	190 (18.1)	
5th quintile	5 (26.3)	344 (32.4)	

Values are mean ± SD or n (%). SIMD, Scottish Index of Multiple Deprivation.

5.4 Cognitive test descriptive statistics

Both baseline and four-year follow-up cognitive test results were used in subsequent analyses and are described here, including inter-correlations, correlations with age, sex and duration of diabetes, and correlations between baseline and year 4.

5.4.1 Baseline

Missing data

A majority of the seven cognitive tests which contributed to *g* (Logical Memory, LM; Faces; Matrix Reasoning, MR; Digit Symbol Coding, DSC; Trail-Making-Test B, TMT-B; Letter Number Sequencing, LNS; Borkowski Verbal Fluency Test, BVFT) were completed by all participants at baseline. Data was missing predominantly due to physical difficulty rather than test refusal and was missing for below 2% of participants on all cognitive tests (for between 18 participants or 1.7% of the total baseline population on Letter-Number Sequencing, and six participants, or 0.06%, on Verbal Fluency).

Because calculation of *g* required completion of all seven of the aforementioned cognitive tests (excluding MHVS and MMSE as an estimate of pre-morbid ability and a screening instrument for dementia, respectively), *g* was derived on the basis of data which had been imputed separately for each of the cognitive tests at both baseline and year 4, as described in Section 4.5.3 of the Method. Consequently, *g* had relatively little missing data at baseline (n=6; 0.6% of baseline population). Data on the MHVS was missing for 19 (1.8%) participants and MMSE data was missing for three (0.03%) participants.

Distributions

Mean ± SD or median (interquartile range) cognitive test performances at baseline are presented in Table 5.8. Note that the minimum values '0' for Logical Memory test of verbal memory and Letter-Number Sequencing test of working memory do not represent missing data; one participant and two participants were willing to perform these two tests respectively, but following test administration were unable to recall any of the target information despite prompting by the tester.

Table 5.8: Baseline cognitive test scores

	N	Mean ± SD or median (interquartile range)	Min	Max
MMSE	1063	29 (28 – 30)	14	30
MHVS	1049	30.93 ± 5.23	9	44
Logical Memory	1050	25.24 ± 8.17	0	46
Faces	1059	65.82 ± 7.88	40	88
Matrix Reasoning	1052	12.81 ± 5.28	3	25
Digit Symbol Coding	1057	49.21 ± 14.77	9	99
Trail-Making (s)	1052	104 (81 – 139)	38	570
Letter Number Sequencing	1048	9.67 ± 2.75	0	19
Verbal Fluency	1060	36.93 ± 12.83	5	79
g	1060	0.00 ± 1.00	-3.36	3.07

Total n = 1066. Data for *g* have been imputed; for remaining cognitive tests are non-imputed. *G* was arbitrarily standardised through principal components analysis, resulting in mean = 0 and SD = 1. This variable was not used to represent four-year cognitive change in *g* in the adjustment method.
MMSE, Mini-Mental-State Examination; MHVS, Mill-Hill Vocabulary Scale ; Verbal Fluency, Borkowski Verbal Fluency Test; Trail-Making, Trail-Making Test-B; s, seconds; max, maximum score; min, minimum score.

Histograms of the cognitive test scores obtained at baseline were also visually inspected (Figure B.37 to B.45 in Appendix B). The distribution for the Trail-Making Test was positively skewed and for MMSE was negatively skewed. Prior to further analyses, the Trail-Making Test was transformed to its natural logarithm to achieve approximately normal distribution (Figure B.46 in Appendix B). Despite skewed distribution, MMSE scores were not log-transformed as the test was not used as a continuous outcome measure, but as a screening instrument for dementia. All remaining cognitive test variables appeared approximately normally distributed.

Inter-correlations amongst baseline cognitive tests

The correlations amongst the cognitive tests and *g* at baseline are shown in Table 5.9. All cognitive tests and *g* correlated significantly with all other cognitive tests. Relatively weaker correlations were found for some of the measures of memory (Logical Memory, Faces); correlations were particularly strong amongst the measures of processing speed (Trail-Making Test-B, Digit Symbol Coding). Note that correlations for Trail-Making Test-B are negative, because in contrast to the remaining cognitive tests, higher values represent a worse performance.

Table 5.9: Bivariate associations amongst baseline cognitive test scores

	LM	Faces	MR	DSC	TMT-B	LNS	BVFT	*g**
MHVS	0.38	0.28	0.45	0.37	-0.37	0.40	0.44	0.58
LM	--	0.24	0.28	0.27	-0.28	0.31	0.25	0.54
Faces		--	0.24	0.29	-0.26	0.20	0.22	0.47
MR			--	0.38	-0.46	0.40	0.36	0.67
DSC				--	-0.63	0.40	0.40	0.75
TMT-B					--	-0.50	-0.39	0.80
LNS						--	0.46	0.72
BVFT							--	0.67

Data for *g* have been imputed, for remaining cognitive tests are non-imputed. *Values for associations of *g* with the seven cognitive tests (except MHVS) are factor loadings on basis of imputed cognitive test data. Values for all individual cognitive tests are correlation coefficients from two-tailed Pearson correlations. All $p<0.001$. MHVS, Mill-Hill Vocabulary Scale; LM, Logical Memory; MR, Matrix Reasoning; DSC, Digit Symbol Coding; TMT-B, Trail-Making-Test B; LNS, Letter Number Sequencing; BVFT, Borkowski Verbal Fluency Test

Associations of baseline cognitive test performance with age, sex and disease duration

Higher age was associated with lower performance on all seven cognitive tests contributing to *g* and with lower *g* (Table 5.10). Disease duration correlated negatively with Faces, Digit Symbol Coding, Trail-Making Test-B, Letter-Number Sequencing, Verbal Fluency and *g*. Performance on the MHVS was unrelated to age or disease duration. Males tended to have higher scores on the MHVS and Matrix Reasoning; females outperformed males on Logical Memory, Faces and Digit Symbol Coding.

Table 5.10: Baseline cognitive test associations with age, sex and disease duration

	Age	Duration of diabetes	Sex		
	Correlation coefficient (p-value)	**Correlation coefficient (p-value)**	**Males**	**Females**	**p-value**
MHVS	0.01 (0.67)	-0.02 (0.60)	**31.37 ± 5.33**	**30.48 ± 5.09**	**0.006**
Logical Memory	**-0.10 (0.001)**	-0.01 (0.72)	**24.45 ± 7.85**	**26.07 ± 8.43**	**0.001**
Faces	**-0.16 (<0.001)**	**-0.12 (<0.001)**	**64.46 ± 7.46**	**67.25 ± 8.08**	**<0.001**
Matrix Reasoning	**-0.17 (<0.001)**	-0.02 (0.44)	**13.47 ± 5.22**	**12.12 ± 5.26**	**<0.001**
Digit Symbol Coding	**-0.21 (<0.001)**	**-0.15 (<0.001)**	**46.80 ± 14.08**	**51.73 ± 15.07**	**<0.001**
Trail-Making (s)	**0.20 (<0.001)**	**0.12 (<0.001)**	108 (83 – 140)	102 (78 – 135)	0.13
Letter Number Sequencing	**-0.16 (<0.001)**	**-0.09 (0.007)**	9.75 ± 2.86	9.59 ± 2.64	0.34
Verbal Fluency	**-0.07 (0.020)**	**-0.09 (0.002)**	37.13 ± 12.87	36.71 ± 12.80	0.60
g	**-0.23 (<0.001)**	**-0.13 (<0.001)**	-0.06 ± 0.98	0.06 ± 1.02	0.06

Analyses are two-tailed Pearson correlations and t-tests. Data for *g* have been imputed, for remaining cognitive tests are non-imputed. MHVS, Mill-Hill Vocabulary Scale; LM, Logical Memory; Trail-Making, Trail-Making-Test-B; LNS, Letter Number Sequencing; Verbal Fluency, Borkowski Verbal Fluency Test. Log-transformed values were used for Trail-Making Test-B and duration of diabetes. Values for age and disease duration are correlation coefficients (p-values); for sex are means ± SD or medians (interquartile range).

Baseline cognitive test performance in people with dementia by year 4

Participants who were identified to suffer from dementia following the year 4 clinic had consistently lower baseline test performance on tests of fluid-type cognitive ability as well as on the MHVS compared with the individuals who remained dementia-free (Table 5.11). This may or may not reflect cognitive deficits due to early signs of dementia, given that the data used to identify subjects with dementia was not limited to the study period. Consequently, participants in the dementia group may have been received diagnosis prior to the baseline assessment.

Table 5.11: Baseline cognitive test scores in the dementia group and the remaining sample

	Dementia (total n=19)		Remaining sample (total n=1047)	
	Total N	**Mean ± SD or median (quartile range)**	**Total N**	**Mean ± SD or median (quartile range)**
MMSE	19	26 (23 – 28)	1044	29 (28 – 30)
MHVS	19	27.05 ± 6.71	1030	31.01 ±5.18
Logical Memory	16	15.81 ± 6.99	1034	25.38 ± 8.11
Faces	19	58.58 ± 5.80	1040	65.95 ± 7.86
Matrix Reasoning	19	8.16 ± 4.15	1033	12.90 ± 5.26
Digit Symbol Coding	18	31.44 ± 14.17	1039	49.52 ± 14.60
Trail-Making (s)	18	177 (154 – 230)	1034	104 (81 – 136)
Letter Number Sequencing	19	6.89 ±3.02	1029	9.72 ± 2.72
Verbal Fluency	19	30.05 ± 11.51	1041	37.05 ± 12.82
g	19	-1.51 ± 0.92	1041	0.03 ± 0.98

Data for *g* have been imputed, for remaining cognitive tests are non-imputed. All group comparisons p<0.001 except Verbal Fluency (p=0.018), MMSE (p=0.002) and MHVS (p=0.001). MMSE, Mini-Mental State Examination; MVHS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; s, seconds.

5.4.2 Four-year follow-up

Missing data

The greatest proportion of missing cognitive data at follow-up was due to non-attendance at the research clinic. Attenders of the year 4 follow-up clinic were found to have consistently higher baseline cognitive test performance compared with non-attenders (Table 5.12). To illustrate, the difference between attenders and no-attenders in baseline *g* was of medium effect size (Cohen's *d* = 0.56) (J. Cohen, 1992), for instance.

Table 5.12: Baseline cognitive test performance of attenders and non-attenders of the year 4 clinic

	Attenders		Non-attenders	
	N	**Mean ± SD or median (interquartile range)**	**N**	**Mean ± SD or median (interquartile range)**
MHVS	820	31.45 ± 5.07	229	29.07 ± 5.37
Logical Memory	822	25.86 ± 7.95	228	23.02 ± 8.57
Faces	827	66.34 ± 7.66	232	63.95 ± 8.40
Matrix Reasoning	822	13.36 ± 5.23	230	10.87 ± 5.00
Digit Symbol Coding	828	50.42 ± 14.34	229	44.81 ± 15.47
Trail-Making (s)	823	101 (79 – 132)	229	124 (94 – 161)
Letter Number Sequencing	822	9.93 ± 2.66	226	8.74 ± 2.89
Verbal Fluency	828	37.77 ± 12.53	232	33.95 ± 13.47
g	829	0.13 ± 0.93	231	-0.44 ±1.11

Data for *g* have been imputed, for remaining cognitive tests are non-imputed. All p<0.001 for group comparisons. MHVS, Mill-Hill Vocabulary Scale; DSC, Digit Symbol Coding; Trail-Making, Trail-Making-Test B; LNS, Letter Number Sequencing; Verbal Fluency, Borkowski Verbal Fluency Test. Log-transformed values were used for Trail-Making Test-B.

Completion of at least part of the cognitive test battery was part of the inclusion criteria for the ET2DS, but this did not apply to the year 4 follow-up. Three participants attending the follow-up clinic did not complete any of the cognitive tests (due to physical disability or refusal). Missing data on the individual cognitive tests (including these three individuals) generally ranged between 30 (3.6% of total year 4 population) on Digit Symbol Coding and Trail-Making Test-B and 7 (0.8%) on Matrix Reasoning. The Letter-Number Sequencing stood out with a relatively higher proportion of data missing for 39 (4.7%) participants, which may be attributable to the year 4 testers offering to omit this test more freely compared with the baseline testers. Additionally, 34 (4.1%) participants did not perform the Simple Reaction Time (SRT) component of the Deary-Liewald Reaction Time Task and 83 (10.0%) participants had missing data on the Choice Reaction Time (CRT) component. Data on the MHVS was missing for eleven (1.3%) participants and MMSE data was missing for seven participants (0.8%).

As at baseline, imputation of cognitive test data for the purpose of calculating *g* resulted in a relatively low proportion of missing data for follow-up *g* (n=8; 1.0% of year 4 sample). Data on a 'four-year change in *g*' variable derived through adjustment of follow-up *g* for baseline *g* was missing for nine attenders (1.1% of year 4 sample).

Distributions

Mean ± SD or median (interquartile range) for year 4 cognitive test performances are presented in Table 5.13 and 5.14. As had also been found for one subject at baseline, one participant attending the year 4 follow-up was unable to recall any of the target material of the Logical Memory test of verbal memory despite prompting, resulting in '0' as the minimum score on this test.

Table 5.13: Year 4 cognitive test scores for attenders

	N	Mean ± SD or median (interquartile range)	Min	Max
MMSE	824	29 (28 – 30)	18	31
MHVS	820	30.71 ± 4.94	10	43
Logical Memory	820	27.28 ± 8.27	0	46
Faces	822	69.23 ± 8.39	44	92
Matrix Reasoning	824	11.56 ± 5.22	2	24
Digit Symbol Coding	801	50.18 ± 14.04	13	103
Trail-Making (s)	801	106 (83 – 142)	43	681
Letter-Number Sequencing	792	8.92 ± 2.89	1	20
Verbal Fluency	822	36.85 ± 12.76	5	97
g	823	0.00 ± 1.00	-2.99	2.92

Total n=831. Data for *g* have been imputed, for remaining cognitive tests are non-imputed. *G* is arbitrarily standardised through principal components analysis, resulting in mean = 0 and SD = 1. This variable was not used to represent four-year cognitive change in *g* in the adjustment method. MMSE, Mini-Mental-State Examination; MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making-Test B; Verbal Fluency, Borkowski Verbal Fluency Test; s, seconds; max, maximum score; min, minimum score.

Table 5.14: Deary-Liewald reaction time at year 4 for attenders

	Simple Reaction Time (SRT)	Choice Reaction Time (CRT)
Correct count	-	39 (38 – 40)
Mean (ms)	305 (276 – 344)	632 ± 123
Standard Deviation (ms)	59 (43 – 90)	157 ± 50

Values are means (SD) or medians (interquartile range). 'CRT correct count' is number of correct trials during CRT (only correct CRT trials were included for calculation of means and standard deviations; maximum n=40); ms, milliseconds.

Histograms of the cognitive test scores obtained at year 4 were also visually inspected (Figure B.47 to B.60 in Appendix B). As at baseline, the distribution for Trail-Making Test-B was positively skewed and for MMSE was negatively skewed. The 'SRT mean' and 'SRT standard deviation' were also positively skewed. 'CRT correct count' describing the number of trials during which a participant responded by pressing the correct key on the keyboard (maximum n=40) was negatively skewed. Prior to further analyses, Trail-Making Test-B, SRT mean, SRT standard deviation and CRT correct count were transformed to their natural logarithms to achieve approximately normal distributions (Figure B.61 to B.64 in Appendix B). Again, MMSE scores were not log-transformed (despite skewed distributions) as the test was again used only as a screening instrument for dementia. All of the remaining cognitive test variables appeared approximately normally distributed.

Inter-correlations amongst follow-up cognitive tests

Correlations amongst cognitive test scores and *g* at year 4 are shown in Table 5.15. Mirroring the pattern found for baseline, scores on Faces and Logical Memory correlated relatively weakly; correlations appeared particularly strong for the tests of processing speed (Trail-Making Test-B; Digit Symbol Coding).

Table 5.15: Bivariate associations amongst year 4 cognitive test scores

	LM	Faces	MR	DSC	TMT-B	LNS	BVFT	*g**
MHVS	0.43	0.29	0.45	0.34	-0.33	0.40	0.42	0.55
LM	--	0.26	0.32	0.37	-0.38	0.37	0.41	0.61
Faces		--	0.29	0.29	-0.31	0.22	0.27	0.51
MR			--	0.44	-0.48	0.46	0.29	0.69
DSC				--	-0.65	0.47	0.41	0.79
TMT-B					--	-0.53	-0.37	0.81
LNS						--	0.41	0.75
BVFT							--	0.62

*Data for *g* have been imputed, for remaining cognitive tests are non-imputed. Values for associations of *g* with the seven cognitive tests (except MHVS) are factor loadings on basis of imputed cognitive test data. Values for all individual cognitive tests are correlation coefficients from two-tailed Pearson correlations. All p<0.001. MHVS, Mill-Hill Vocabulary Scale; LM, Logical Memory; MR, Matrix Reasoning; DSC, Digit Symbol Coding; TMT-B, Trail-Making-Test B; LNS, Letter Number Sequencing; BVFT, Borkowski Verbal Fluency Test. Log-transformed values were used for Trail-Making Test-B.

All of the CRT measures correlated with one another and with performance on the SRT, with the exception of a non-significant association between CRT correct count and SRT mean (Table 5.16). Compared with the remaining variables, effect sizes were relatively small for associations of CRT correct count. A weak positive association between CRT correct count and CRT mean could reflect a trade-off between speed and accuracy on the task. The different SRT measures were all highly correlated.

Table 5.16: Univariate associations amongst Simple Reaction Time (SRT) and Choice Reaction Time (CRT) measures

	CRT correct count	CRT mean	CRT SD	SRT mean
CRT mean	0.10 (0.006)	-		
CRT SD	0.07 (0.051)	0.62 (<0.001)	-	
SRT mean	-0.02 (0.623)	0.50 (<0.001)	0.22 (<0.001)	-
SRT SD	-0.08 (0.030)	0.28 (<0.001)	0.18 (<0.001)	0.70 (<0.001)

Values are correlation coefficients from two-tailed Pearson correlations (p-values). SRT, simple reaction time; CRT, choice reaction time; 'SRT SD', standard deviation in SRT component; 'CRT SD', standard deviation in CRT component; 'CRT correct count' is number of correct trials during CRT (only correct CRT trials were used to calculate means and standard deviations; maximum n = 40). SRT mean, SRT SD and CRT correct count were log-transformed. Higher values of SRT mean and CRT mean represent worse performance, whereas higher values of CRT correct count indicate better performance.

All further analyses on reaction time were carried out only for SRT mean and CRT mean as the main reaction time variables of interest. The associations of these measures with performance on the remaining cognitive tests at year 4 are shown in Table 5.17. Individuals who performed well on the CRT and SRT tended to also perform well on the remaining cognitive tests and *g*.

Table 5.17: Simple and Choice Reaction Time associations with year 4 cognitive test scores

	CRT mean	SRT mean
MHVS	-0.17	-0.20
Logical Memory	-0.16	-0.13
Faces	-0.17	-0.14
Matrix Reasoning	-0.25	-0.22
Digit Symbol Coding	-0.39	-0.28
Trail-Making	0.33	-0.30
Letter-Number Sequencing	-0.25	-0.16
Verbal Fluency	-0.20	-0.19
g	-0.38	-0.30

Analyses are two-tailed Pearson correlations. Trail-Making and SRT mean were log-transformed. Data for *g* have been imputed, for remaining cognitive tests are non-imputed. Values are correlation coefficients. All p<0.001. Higher values of Trail-Making, SRT mean and CRT mean represent worse performance; for all remaining cognitive tests higher values represent better performance. Trail-Making Test-B and SRT mean were log-transformed. MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making-Test B; Verbal Fluency, Borkowski Verbal Fluency Test; SRT, simple reaction time; CRT, choice reaction time.

Associations of follow-up cognitive test performance with age, sex and disease duration

Similar to baseline, age correlated negatively with performance on all individual cognitive tests contributing to *g*, except Verbal Fluency (Table 5.18). Longer disease duration was associated with lower performance on Faces, Digit Symbol Coding, Trail-Making Test-B, Verbal Fluency and *g*. Males outperformed females on the MHVS and Matrix Reasoning, and had lower mean reaction time (i.e. better performance) on both components of the reaction time task compared with females; females on average scored higher or performed better than males on Logical Memory, Faces, Digit Symbol Coding, Trail-Making Test-B and *g*. None of the remaining associations of cognitive test performance with age, disease duration or sex reached statistical significance. The non-significant relationship between MHVS and age as well as disease duration supports the measure as an estimate of crystallised-type cognitive ability which is relatively resistant cognitive decline.

Table 5.18: Year 4 cognitive test associations with age, sex and disease duration

	Age	Duration of diabetes	Sex		
	Correlation coefficient (p-value)	**Correlation coefficient (p-value)**	**Males**	**Females**	**p-value**
MHVS	<0.01 (0.99)	-0.01 (0.72)	**31.26 ± 4.88**	**30.12 ±4.95**	**0.001**
Logical Memory	**-0.12 (0.001)**	-0.03 (0.40)	**26.32 ± 8.47**	**28.29 ± 7.93**	**0.001**
Faces	**-0.20 (<0.001)**	**-0.11 (0.003)**	**68.02 ± 7.88**	**70.51 ± 8.72**	**<0.001**
Matrix Reasoning	**-0.17 (<0.001)**	-0.07 (0.07)	**12.13 ± 5.21**	**10.96 ±5.16**	**0.001**
Digit Symbol Coding	**-0.22 (<0.001)**	**-0.09 (0.008)**	**47.99 ± 13.73**	**52.53 ± 14.01**	**<0.001**
Trail-Making (s)	**0.25 (<0.001)**	**0.08 (0.020)**	108 (85 – 145)	105 (81 – 136)	0.052
Letter-Number Sequencing	**-0.16 (<0.001)**	-0.06 (0.09)	8.98 ± 2.94	8.86 ± 2.85	0.56
Verbal Fluency	-0.05 (0.13)	**-0.09 (0.008)**	36.76 ±12.92	36.95 ± 12.60	0.83
g	**-0.24 (<0.001)**	**-0.11 (0.002)**	**-0.07 ± 0.99**	**0.07 ± 1.01**	**0.039**
CRT mean (ms)	**0.15 (<0.001)**	0.04 (0.305)	**624 ± 122**	**642 ± 122**	**0.050**
SRT mean (ms)	**0.16 (<0.001)**	0.04 (0.232)	**297 (269 – 342)**	**310 (284 – 347)**	**0.011**

Analyses are two-tailed Pearson correlations and t-tests. MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making-Test B;; Verbal Fluency, Borkowski Verbal Fluency Test; SRT, Simple Reaction Time; CRT, Choice Reaction Time; ms, milliseconds; s, seconds. Log-transformed values were used for Trail-Making, SRT mean and duration of diabetes. Values for age and disease duration are correlation coefficients (p-values); for sex are means ± SD or medians (interquartile range). Data for *g* have been imputed, for remaining cognitive tests are non-imputed. Higher values for Trail-Making Test-B, CRT mean and SRT mean represent worse performance and for the remaining cognitive tests and *g* represent better performance.

5.4.3 Correlations between cognitive test scores at baseline and follow-up

All cognitive tests showed high stability of individual differences between baseline and year 4 (Table 5.19). Performance on the MHVS was the most stable of all cognitive tests, further supporting its usefulness to estimate crystallised-type ability, although some severe cases of decline (e.g., from 31 out of 43 at baseline to 19 at year 4) or strong improvements (e.g., from 11 at baseline to 19 at year 4) were observed for this test (these were not explained by non-nativeness, error in data entry or dementia). The tests of memory (Letter-Number Sequencing, Logical Memory, Faces) correlated relatively weakly between the two time points compared with the remaining cognitive tests (although still with large effect sizes); tests of speed of processing and executive function (Verbal Fluency, Digit Symbol Coding, Trail-Making Test-B) as well as *g* correlated particularly strongly. For *g* as the main cognitive variable of interest, the correlation between baseline and follow-up scores is illustrated in Figure 5.2.

Table 5.19: Univariate associations of baseline with year 4 cognitive test scores

	Correlation coefficient
MHVS	0.89
g	0.85
Verbal Fluency	0.81
Digit Symbol Coding	0.75
Trail-Making	0.69
Matrix Reasoning	0.67
Logical Memory	0.63
Faces	0.61
Letter-Number Sequencing	0.54

Analyses were two-tailed Pearson correlations. All $p<0.001$. MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making-Test B; Verbal Fluency, Borkowski Verbal Fluency Test. Log-transformed values were used for Trail-Making. Data for *g* have been imputed, for remaining cognitive tests are non-imputed.

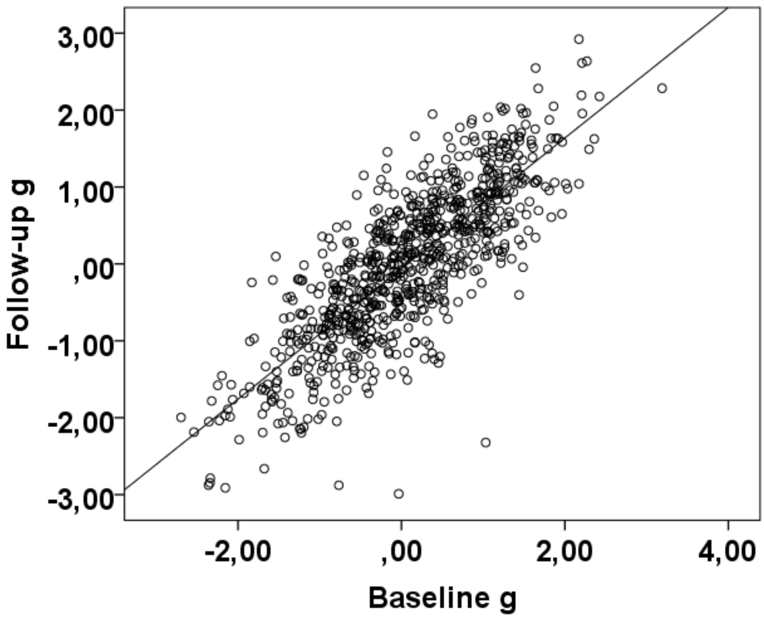

Figure 5.2: Scatterplot showing the association of baseline *g* and follow-up *g* for attenders

5.4.4 Change in cognitive test scores during follow-up

Change in cognitive test performance between baseline and year 4 follow-up was analysed for attenders of the year 4 clinic. Mean scores decreased significantly on MHVS, Matrix Reasoning, Letter-Number Sequencing, Verbal Fluency and *g* and the mean time required by participants to complete the Trail-Making Test-B increased (Table 5.20). Performance on Faces and Logical Memory improved although this does not necessarily imply improved ability, because memory for the specific stimuli between the clinic visits could potentially have affected scores. A decline in Digit Symbol Coding performance approached statistical significance.

As described in section 4.5.2, original data for the seven cognitive tests contributing to *g* were used for all analyses presented in this thesis. A comparison of the four-year change in scores on the individual cognitive tests using original, non-imputed data (Table 5.20) with the same analysis using imputed data (Appendix C) shows that findings were close to identical when imputed data and when originally measured data were used.

Table 5.20: Cognitive change between baseline and year 4 for attenders

		Baseline	Year 4	
	N	Mean ± SD or median (interquartile range)	Mean ± SD or median (interquartile range)	p-value
MHVS	810	31.53 ± 5.02	30.70 ± 4.95	<0.001
Logical Memory	812	25.88 ± 7.96	27.37 ± 8.14	<0.001
Faces	819	66.42 ± 7.63	69.21 ± 8.39	<0.001
Matrix Reasoning	816	13.38 ± 5.22	11.58 ± 5.22	<0.001
Digit Symbol Coding	801	50.86 ± 14.08	50.18 ± 14.04	0.06
Trail-Making (s)	795	101 (79 – 132)	106 (83 – 142)	<0.001
Letter-Number Sequencing	784	10.01 ± 2.64	8.93 ± 2.89	<0.001
Verbal Fluency	819	37.84 ± 12.53	36.87 ± 12.77	<0.001
g	822	0.07 ± 0.95	-0.06 ± 1.04	<0.001

MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making-Test B; Verbal Fluency, Borkowski Verbal Fluency Test; s, seconds. Log-transformed values were used for analyses of Trail-Making. Data for *g* have been imputed; for individual cognitive tests are non-imputed. The *g* baseline and *g* follow-up variables were standardised on the same population to allow comparison.

5.4.5 Unviariate associations between demographic variables, estimated peak pre-morbid ability and four-year change in *g*

An analysis of variance (ANOVA) revealed a statistically significant association of SIMD quintile with peak pre-morbid ability estimated at baseline (MHVS) ($F(4,1044) = 26.26$; $p<0.001$). Pairwise comparison then determined that the first (most deprived) quintile had a significantly lower mean MVHS (28.69, 95% CI 27.81, 29.57) compared with the third (mean 30.12, 95% CI 29.40, 30.83), fourth (mean 31.57, 95% CI 30.84, 32.28) and fifth (least deprived; mean 32.84, 95% CI 32.31, 33.38) quintile. The second (mean 29.29, 95% CI 28.60, 29.97) as well as the third quintile groups both had significantly lower mean MHVS scores compared with the fourth and fifth quintiles. Subjects in the fifth quintile (highest socioeconomic status) had significantly higher mean MHVS scores compared with all of the remaining SIMD groups (all $p<0.05$). This finding is illustrated in Figure 5.3.

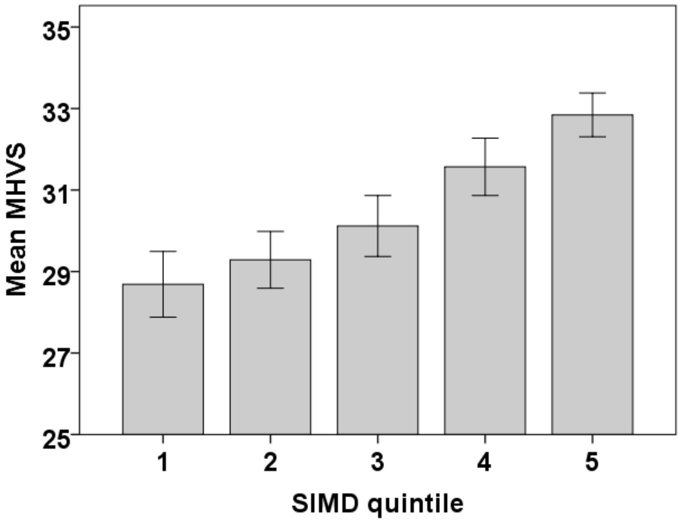

Figure 5.3: Mean baseline MHVS scores according to SIMD quintile (error bars show 95%CI)

Unadjusted linear regression analyses investigated the relationship of age, sex, disease duration and MHVS with the four-year change in cognitive function. To summarise, advanced age and a lower score on the MHVS (indicating lower peak pre-morbid ability) were both associated with a steeper decline in cognitive ability between baseline and year 4 assessments in terms of all individual cognitive tests as well as global ability measured by *g* (Table 5.22). For analyses of MHVS, associations were of relatively largest effect size for *g*, and for working and verbal memory (Letter-Number Sequencing; Logical Memory) and reasoning (Matrix Reasoning). For age, findings of a relationship with a steeper decline appeared relatively strongest for non-verbal memory (Faces) and for executive function (Trail-Making Test-B). Sex and disease duration were relatively unrelated to the change in cognitive test performance between baseline and year 4, both in terms of individual tests and *g*. An exception was a steeper decline in Matrix Reasoning score in individuals with longer disease duration.

Table 5.22: Univariate associations of baseline Mill-Hill Vocabulary Score, age, sex and disease duration with four-year change in cognitive function

	Age	Sex	Duration of diabetes	MHVS
Logical Memory	**-0.08 (0.004)**	0.05 (0.10)	-0.04 (0.14)	**0.23 (<0.001)**
Faces	**-0.13 (<0.001)**	0.05 (0.06)	0.03 (0.30)	**0.14 (<0.001)**
Matrix Reasoning	**-0.08 (0.004)**	-0.02 (0.45)	**-0.06 (0.015)**	**0.19 (<0.001)**
Digit Symbol Coding	**-0.11 (<0.001)**	0.03 (0.16)	-0.02 (0.45)	**0.11 (<0.001)**
Trail-Making (s)	**0.15 (<0.001)**	-0.04 (0.10)	0.04 (0.10)	**-0.12 (<0.001)**
Letter-Number Sequencing	**-0.10 (0.002)**	0.00 (0.99)	-0.04 (0.21)	**0.23 (<0.001)**
Verbal Fluency	**-0.05 (0.009)**	0.04 (0.08)	-0.03 (0.12)	**0.08 (<0.001)**
g	**-0.11 (0.002)**	0.04 (0.20)	-0.05 (0.16)	**0.17 (<0.001)**

Analyses are linear regression analyses of follow-up cognitive test scores adjusted for baseline scores. Values are correlation coefficients (p-values). MHVS measured at baseline. MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; data for *g* has been imputed, for individual cogntiive tests is non-imputed. Higher values of Trail-Making Test-B indicate worse performance. Duration of diabetes and Trail-Making Test-B are log-transformed. Sex coded as 1 = male; 2 = female. For analyses of age and sex, n = 784 to 822; for analyses of duration of diabetes, n = 777 to 815; for analyses of MHVS, n = 777 to 811.

Analyses of the relationship of education and social class with four-year cognitive change were restricted to *g* (rather than the seven individual cognitive tests) for reasons of brevity. As shown in Table 5.23 and illustrated in Figure 5.4, subjects with a lower socioeconomic status tended to experience steeper cognitive decline between baseline and year 4, although post-hoc pairwise comparison showed that only the difference in four-year decline in *g* between the fifth and the first (p=0.001) as well as the third quintile (p=0.004) reached statistical significance. A similar trend short of statistical significance was observed for associations of the level of education of participants with the four-year change in *g* (Table 5.23).

Table 5.23: Univariate associations of education and deprivation with four-year change in *g*

	Mean standardised residual of follow-up *g* adjusted for baseline *g* (95% CI)	p-value for trend
Education		0.07
University degree	0.13 (-0.04, 0.92)	
Professional qualification	0.05 (-0.07, 0.18)	
Secondary school	-0.06 (-0.16, 0.03)	
Primary school	-0.66 (-1.53, 0.22)	
SIMD rank		**<0.001**
1st quintile	**-0.22 (-0.42, -0.02)**	
2nd quintile	**-0.16 (-0.32, 0.00)**	
3rd quintile	**-0.11 (-0.27, 0.06)**	
4th quintile	**0.03 (-0.13, 0.19)**	
5th quintile	**0.19 (0.07, 0.30)**	

Analyses are analyses of variance (ANOVA) of follow-up *g* adjusted for baseline *g*. CI, confidence interval; SIMD, Scottish Index of Multiple Deprivation.

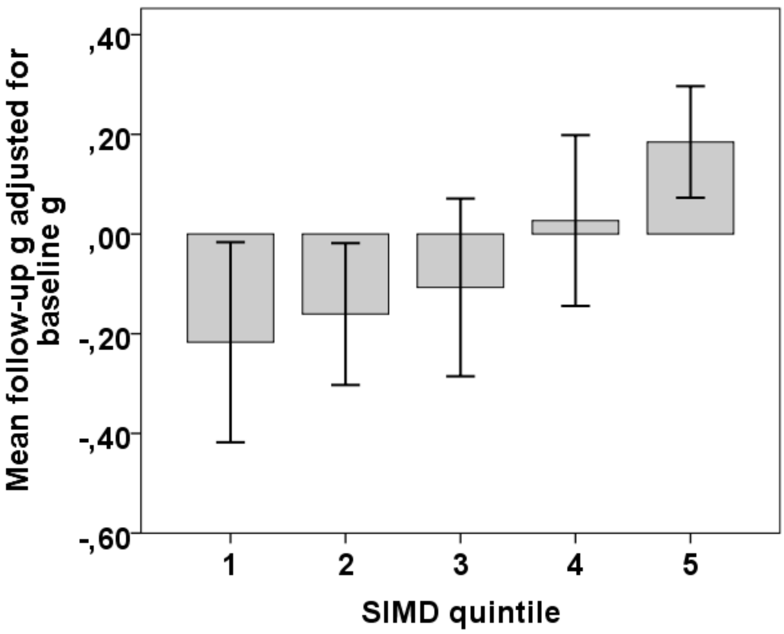

Figure 5.4: Mean four-year change in *g* according to SIMD quintile (error bars show 95% CI)

Risk factors for cognitive decline in older people with type 2 diabetes

Volume II:

Results II, III, IV, Discussion, Bibliography and Appendices

Insa Feinkohl

Presented for the degree of Doctor in Philosophy by Research

University of Edinburgh

Autumn 2013

Declaration

I, Insa Feinkohl, declare that this thesis is my own composition. The work presented here has not been submitted for any other degree or professional qualification.

The Edinburgh Type 2 Diabetes Study, which provided the data for all analyses presented here, had already completed the baseline and year 1 clinics by the time I joined the study, but I was involved in the collection of cognitive data at the year 4 follow-up clinic in 2010/2011. Therefore, all of the baseline clinical variables and the baseline cognitive test data used for the purpose of the analyses presented in this thesis were collected, cleaned and (in some cases) manipulated through the efforts of other members of the research team. However, all of the statistical analyses on the basis of this data were performed by myself.

Some of the findings from analyses presented in this thesis were published in scientific journals. The manuscripts are included in the Appendix with permission from Diabetes Care®.

Signed: ______________________

Date: 05/05/2014

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Abbreviations

ABI	ankle brachial pressure index
BVFT	Borkowski Verbal Fluency Test
CI	confidence interval
cIMT	carotid intima-media thickness
CRP	C-reactive protein
CVD	cardiovascular disease
DR	diabetic retinopathy
DSC	Digit Symbol Coding
Faces	Faces test
HADS	Hospital Anxiety and Depression Scale
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IL-6	interleukin-6
LM	Logical Memory
LNS	Letter-Number Sequencing
MI	myocardial infarction
MMSE	Mini-Mental-State Examination
MR	Matrix Reasoning
MVD	macrovascular disease
NART	National Adult Reading Test
NT-proBNP	N-terminal pro-brain natriuretic peptide
OR	odds ratio
PAD	peripheral arterial disease
RCT	randomised controlled trial
SD	standard deviation
SES	socioeconomic status
SH	severe hypoglycaemia
St. Error	standard error
TIA	transient ischaemic attack
TMT-B	Trail-Making Test-B
TNF-α	tumor necrosis factor-α
WAIS	Wechsler Adult Intelligence Scale
WMS	Wechsler Memory Scale

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Chapter 6: Results II: Macrovascular disease and cognition

This chapter presents results on the association between baseline macrovascular disease (MVD) and cognition in the ET2DS. Variables representing MVD are further categorised as measures of 'symptomatic' disease (myocardial infarction, angina, stroke, transient ischaemic attack, peripheral arterial disease of the lower limbs) and 'subclinical' disease (NT-proBNP, ankle brachial index and carotid intima medial thickness). Cognitive outcomes for these analyses include vocabulary (MHVS measured at baseline; an indicator of peak prior cognitive ability), late life cognitive ability (cognitive function at year 4), estimated relative lifetime cognitive change (year 4 cognitive ability adjusted for MHVS), four-year cognitive change (year 4 cognitive ability adjusted for baseline ability) and presence of dementia by year 4 (n=19). In order to determine whether or not the findings presented in this chapter were driven by patients with dementia, selected analyses (those of MHVS and of four-year cognitive change – the main outcome of interest) were repeated with their exclusion. Results from these analyses, which did not alter statistical significance or effect sizes compared with the analyses presented in the current chapter, are shown in Appendix E. In addition to potential confounders included in each analysis, data from the Scottish Index of Multiple Deprivation (SIMD) was controlled for in a final modelling step in analyses of MHVS and of *g* in order to evaluate the role of socioeconomic status in associations of estimated pre-morbid cognitive ability with late-life risk of macrovascular disease as well as between late-life macrovascular disease and late-life cognition.

6.1 Macrovascular disease and estimated peak pre-morbid ability

To determine the association of macrovascular disease with estimated peak pre-morbid ability at baseline, linear regression analysis of MHVS on each macrovascular risk factor was used. For each symptomatic disease category, comparisons were made both against subjects without disease in that specific category, and against subjects without any symptomatic disease (n=673). A lower peak pre-morbid ability, as estimated by lower baseline MHVS scores, was significantly associated with the presence of stroke, myocardial infarction (MI) and angina, with relatively largest effect sizes for the association with angina (Table 6.1; Figure 6.1). The restriction of comparisons to individuals who were free of any symptomatic disease increased effect sizes throughout. ABI was significantly associated with MHVS: each standard deviation decrease in ABI was associated with a 0.09 standard deviation decrease in MHVS. Associations of MHVS with NT-proBNP and cIMT were not statistically significant. Adjustment for SIMD rendered all associations except that for angina statistically non-significant. For angina, the association was also severely weakened (close to halved) in this modelling step. Overall, the findings show that subjects with a lower (estimated) cognitive ability in young adulthood were at an increased risk of having experienced a stroke and to be suffering from coronary heart disease and peripheral atherosclerotic disease at the time of attending the baseline clinic of the ET2DS, but the findings were largely driven by individual differences in SES. It may be plausible that subjects with a relatively lower socioeconomic status were exposed to environments which did not stimulate the accumulation of cognitive abilities (for instance, people from lower socioeconomic status may have been more concerned with survival than with cognitively engaging activities such as reading). At the same time, such environments may have had detrimental effects on the risk of late-life macrovascular disease, although of course the specific relationship of socioeconomic status, pre-morbid ability and later atherosclerosis does not become apparent on the basis of the present data. A mediatory role of socioeconomic status may also be possible, with relatively lower cognitive ability in young adulthood predisposing to a lower socioeconomic status and then to an increased risk of late-life macrovascular disease. The survival of angina associations with MHVS following adjustment for covariates including socioeconomic status could potentially be attributed to a relatively higher statistical power due to a relatively large proportion of the sample suffering from angina.

Table 6.1: Linear regression analyses of associations between macrovascular disease and MHVS at baseline

	Stroke (n= 62)	TIA (n=31)	MI (n=150)	Angina (n=298)	PAD (n=65)	NT-proBNP	Ankle brachial index	Carotid IMT
Analysis of all participants[a]								
Model 1: Adjusted for age, sex	**-0.06 (0.042)**	-0.01 (0.85)	-0.06 (0.053)	**-0.11 (<0.001)**	-0.01 (0.78)	-0.06 (0.06)	**0.09 (0.006)**	0.02 (0.55)
Model 2: Adjusted for age, sex, SIMD	-0.04 (0.22)	0.01 (0.78)	-0.03 (0.30)	**-0.07 (0.031)**	0.00 (0.91)	-0.03 (0.28)	0.06 (0.06)	0.03 (0.33)
Comparison with participants free of any symptomatic MVD (n=673)								
Model 1: Adjusted for age, sex	**-0.10 (0.005)**	-0.03 (0.44)	**-0.10 (0.007)**	**-0.14 (<0.001)**	-0.04 (0.28)	--	--	--
Model 2: Adjusted for age, sex, SIMD	-0.07 (0.07)	-0.01 (0.90)	-0.06 (0.10)	**-0.09 (0.007)**	-0.02 (0.63)			

Outcome measure is baseline MHVS. Analyses adjusted for age, sex. Values are standardised β coefficients (p-values). [a]for symptomatic MVD predictors, analyses compared to all other participants. MHVS, Mill-Hill Vocabulary Scale; MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease; NT-proBNP, N-terminal pro-brain natriuretic peptide; carotid IMT, carotid intima-media thickness, SIMD, Scottish Index of Multiple Deprivation (quintiles). NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917

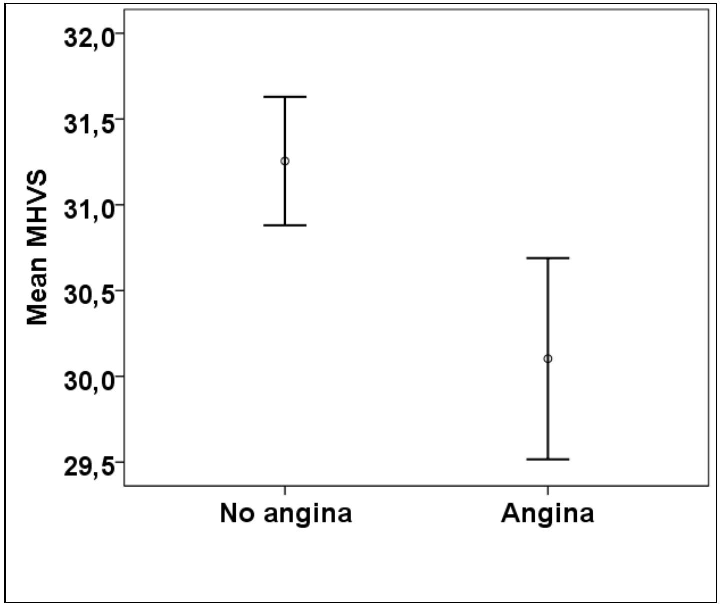


Figure 6.1: Mean MHVS scores in subject with and those without angina at baseline (error bars show 95% CI)

6.2 Macrovascular disease and cognitive function at year 4

6.2.1 Symptomatic disease

The associations of symptomatic macrovascular disease at baseline with the level of cognitive function at year 4 were investigated in multiple linear regression analyses of the global ability factor *g* and scores on the individual cognitive tests at year 4 on each of the five measures of symptomatic macrovascular disease. For each model, an initial step controlled for age and sex, followed by additional adjustment for a range of established cardiovascular risk factors. Results for *g* and the majority of the individual tests are shown in Table 6.2 with results for reaction time in Table 6.3. As for other analyses in this chapter, comparisons for each symptomatic disease category were made both against subjects without disease in that specific category, and against subjects without any symptomatic disease (n=538). For *g*, which was the main cognitive outcome of interest, SIMD was controlled for in a final modelling step to determine the independence of findings from socioeconomic status.

A prior history of stroke was significantly associated with lower performance or trends for lower performance on a majority of cognitive tests and *g* at year 4, but the results for TIA tended to be non-significant (Table 6.2; Table 6.3). Consistent with MI and angina as manifestations of coronary heart disease, patterns of findings were similar for each of these disease categories, with both associated with lower working memory (Letter-Number Sequencing), executive function (Trail-Making Test-B), reasoning (Matrix Reasoning) and processing speed (Digit Symbol Coding). With exception of an association with longer mean Choice Reaction Time latencies, the presence of PAD was unrelated to cognitive function at year 4 in the analysis of the entire sample; statistically significant associations were present only when comparisons were made with participants free of all symptomatic MVD.

Almost all of the significant associations of symptomatic macrovascular predictors with cognitive test scores and *g* survived adjustment for conventional vascular risk factors, but were weakened following adjustment for SIMD to the extent that all associations with *g* except those for stroke, and for MI and angina (when compared with MVD-free individuals) became statistically non-significant. This also reflects the observation that generally, results were similar when comparing the disease categories to all of the remaining participants, and when restricting the comparison to individuals free of all symptomatic MVD, although in the latter analyses effect sizes tended to be increased and significance levels improved.

Associations of baseline symptomatic macrovascular disease with the risk of dementia diagnosis by year 4 were investigated using age-and sex-adjusted logistic regression analyses of presence of dementia on each of the measures of symptomatic macrovascular disease. A baseline history of stroke (age- and sex-adjusted OR 1.65, 95% CI 0.36, 7.57, p=0.52) and angina (age- and sex-adjusted OR 0.68, 95% CI 0.24, 1.96, p=0.48) were found to be both unrelated to dementia diagnosis, but trends were observed for increased prevalence of TIA and MI in the dementia group. Two individuals with TIA (6.5%) suffered from dementia compared with 17 individuals without TIA (1.6%; age- and sex-adjusted OR 3.89, 95% CI 0.83, 18.23, p=0.09). A history of MI was prevalent in 31.6% (n=6) of the subjects in the dementia group (31.6%) whereas only 13.8% (n=144) of the remaining sample had suffered MI (age- and sex-adjusted OR 2.40, 95% CI 0.85, 6.79, p=0.10). None of the 19 individuals with dementia had PAD at baseline.

Table 6.2: Linear regression analyses of associations between symptomatic macrovascular disease and cognitive function (*g* and individual cognitive tests) at year 4

	Stroke (n=44)		TIA (n=27)		MI (n=111)		Angina (n=222)		PAD (n=53)	
	Vs. no stroke	**Vs. no symptomatic MVD**	**Vs. no TIA**	**Vs. no symptomatic MVD**	**Vs. no MI**	**Vs. no symptomatic MVD**	**Vs. no angina**	**Vs. no symptomatic MVD**	**Vs. no PAD**	**Vs. no symptomatic MVD**
Logical Memory										
Age, sex adjusted	**-0.07 (0.040)**	**-0.11 (0.006)**	0.01 (0.75)	0.00 (0.97)	-0.02 (0.50)	-0.06 (0.12)	**-0.09 (0.010)**	**-0.11 (0.003)**	-0.05 (0.18)	**-0.08 (0.054)**
Multi-adjusted[a]	**-0.08 (0.031)**	**-0.12 (0.004)**	0.01 (0.75)	-0.01 (0.88)	-0.03 (0.41)	-0.07 (0.07)	**-0.10 (0.005)**	**-0.12 (0.001)**	-0.05 (0.17)	**-0.09 (0.038)**
Faces										
Age, sex adjusted	-0.06 (0.11)	**-0.09 (0.035)**	-0.04 (0.25)	-0.07 (0.12)	-0.04 (0.27)	-0.07 (0.06)	-0.05 (0.17)	**-0.07 (0.052)**	-0.06 (0.07)	**- 0.09 (0.031)**
Multi-adjusted[a]	-0.05 (0.17)	-0.08 (0.06)	-0.04 (0.26)	-0.07 (0.12)	-0.04 (0.32)	-0.07 (0.07)	-0.05 (0.19)	-0.07 (0.06)	-0.06 (0.09)	**0.09 (0.025)**
Letter Number										
Age, sex adjusted	**-0.07 (0.051)**	**-0.13 (0.002)**	-0.03 (0.38)	-0.06 (0.17)	-0.07 (0.06)	**-0.11 (0.005)**	**-0.08 (0.020)**	**-0.10 (0.005)**	-0.06 (0.11)	**-0.09 (0.033)**
Multi-adjusted[a]	-0.07 (0.06)	**-0.11 (0.011)**	-0.03 (0.34)	-0.06 (0.18)	**-0.07 (0.050)**	**-0.10 (0.015)**	**-0.09 (0.016)**	**-0.11 (0.006)**	-0.06 (0.10)	**-0.09 (0.032)**
Trail-Making										
Age, sex adjusted	**0.14 (<0.001)**	**0.21 (<0.001)**	0.01 (0.68)	0.04 (0.41)	0.07 (0.06)	**0.09 (0.019)**	**0.07 (0.053)**	**-0.09 (0.018)**	0.05 (0.13)	**0.09 (0.024)**
Multi-adjusted[a]	**0.14 (<0.001)**	**0.18 (<0.001)**	0.01 (0.71)	0.03 (0.47)	0.06 (0.07)	**0.10 (0.09)**	0.07 (0.07)	**0.09 (0.021)**	0.05 (0.17)	0.07 (0.08)
Verbal Fluency										
Age, sex adjusted	**-0.11 (0.002)**	**-0.15 (<0.001)**	0.01 (0.75)	-0.01 (0.89)	-0.02 (0.50)	-0.06 (0.17)	-0.04 (0.27)	**-0.06 (0.09)**	-0.04 (0.21)	-0.07 (0.10)
Multi-adjusted[a]	**-0.11 (0.003)**	**-0.15 (0.001)**	0.01 (0.83)	-0.01 (0.85)	-0.03 (0.40)	-0.06 (0.12)	-0.04 (0.22)	-0.07 (0.06)	-0.05 (0.16)	-0.08 (0.07)
Digit Symbol Coding										
Age, sex adjusted	**-0.15 (<0.001)**	**-0.22 (<0.001)**	-0.02 (0.59)	-0.04 (0.34)	**-0.09 (0.013)**	**-0.12 (0.002)**	**-0.13 (<0.001)**	**-0.15 (<0.001)**	-0.01 (0.70)	-0.06 (0.15)
Multi-adjusted[a]	**-0.14 (<0.001)**	**-0.19 (<0.001)**	-0.01 (0.67)	-0.04 (0.37)	**-0.08 (0.019)**	**-0.12 (0.003)**	**-0.14 (<0.001)**	**-0.15 (<0.001)**	0.00 (0.99)	-0.03 (0.46)
Matrix Reasoning										
Age, sex adjusted	-0.06 (0.06)	**-0.10 (0.020)**	**-0.11 (0.002)**	**-0.14 (0.001)**	**-0.10 (0.005)**	**-0.13 (0.001)**	**-0.07 (0.041)**	**-0.09 (0.013)**	0.00 (0.97)	-0.02 (0.57)
Multi-adjusted[a]	-0.06 (0.08)	**-0.09 (0.033)**	**-0.11 (0.002)**	**-0.14 (0.001)**	**-0.09 (0.009)**	**-0.11 (0.005)**	-0.07 (0.07)	**-0.08 (0.034)**	0.01 (0.81)	-0.01 (0.79)
g										
Age, sex adjusted	**-0.16 (<0.001)**	**-0.22 (<0.001)**	-0.04 (0.29)	-0.07 (0.08)	**-0.09 (0.013)**	**-0.14 (<0.001)**	**-0.11 (0.002)**	**-0.14 (<0.001)**	-0.06 (0.10)	**-0.10 (0.011)**
Multi-adjusted[a]	**-0.15 (<0.001)**	**-0.21 (<0.001)**	-0.04 (0.30)	-0.07 (0.10)	**-0.08 (0.017)**	**-0.14 (0.001)**	**-0.11 (0.002)**	**-0.14 (<0.001)**	-0.05 (0.13)	**-0.10 (0.020)**
+SIMD[b]	**-0.13 (<0.001)**	**-0.17 (<0.001)**	-0.01 (0.71)	-0.03 (0.42)	-0.05 (0.11)	**-0.10 (0.014)**	-0.06 (0.08)	**-0.09 (0.013)**	-0.04 (0.21)	-0.07 (0.07)

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. For each independent variable, comparisons are made with the respective remaining sample and with participants free of any symptomatic MVD (n=538).MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease; SIMD, Scottish Index of Multiple Deprivation (quintiles); Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; Letter Number, Letter Number Sequencing.

[a]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit

[b]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles).

Table 6.3: Linear regression analyses of associations between symptomatic macrovascular disease and reaction time at year 4

	Simple Reaction Time (SRT) mean		Choice Reaction Time (CRT) mean	
	Age, sex adjusted	**Multi-adjusted[a]**	**Age, sex adjusted**	**Multi-adjusted[a]**
Stroke **vs. no stroke**	**0.09 (0.011)**	**0.09 (0.011)**	0.05 (0.19)	0.05 (0.19)
vs. no symptomatic MVD	**0.11 (0.001)**	**0.11 (0.008)**	0.05 (0.32)	0.07 (0.14)
TIA **vs. no TIA**	0.01 (0.80)	0.01 (0.75)	0.00 (0.94)	0.01 (0.90)
vs. no symptomatic MVD	0.02 (0.60)	0.02 (0.59)	0.02 (0.70)	0.02 (0.68)
MI **vs. no MI**	-0.03 (0.48)	-0.03 (0.46)	-0.02 (0.55)	-0.02 (0.55)
vs. no symptomatic MVD	0.00 (0.93)	0.00 (0.99)	0.01 (0.80)	0.01 (0.87)
Angina **vs. no angina**	0.04 (0.23)	0.04 (0.30)	0.07 (0.08)	0.06 (0.09)
vs. no symptomatic MVD	0.05 (0.17)	0.04 (0.27)	0.08 (0.06)	0.07 (0.09)
PAD **vs. no PAD**	0.01 (0.88)	-0.01 (0.87)	**0.08 (0.020)**	**0.08 (0.023)**
vs. no symptomatic MVD	0.01 (0.87)	0.01 (0.87)	**0.09 (0.046)**	**0.11 (0.010)**

Values are standardised β coefficients (p-values). Simple Reaction Time mean is log-transformed. MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease.

[a]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic.

Stroke n=44; TIA, n=27; MI, n=111; angina n=22; PAD n=53; no symptomatic MVD n=538.

6.2.2 Subclinical disease

The associations of ABI, cIMT and NT-proBNP with level of cognitive function at year 4 were investigated using multiple linear regression analyses of *g* and scores on the individual cognitive tests at year 4 on ABI, cIMT and NT-proBNP. Again, initial adjustment was made for age and sex, followed by additional adjustment for a range of established cardiovascular risk factors, and, for *g*, for SIMD quintiles measuring participants' socioeconomic status as well as for a prior history of stroke in separate steps. Results for *g* and the majority of the individual tests are shown in Table 6.4, with results for reaction time in Table 6.5.

All three subclinical measures were relatively weakly but significantly associated with follow-up *g*. Each standard deviation lower ABI, higher cIMT and higher NT-proBNP were associated with a 0.12 standard deviation, a 0.09 standard deviation and a 0.10 standard deviation lower *g*, respectively. In terms of individual cognitive tests, findings were largely similar for NT-proBNP and ABI, although effect sizes overall were somewhat larger for ABI than for NT-proBNP. Higher NT-proBNP and lower ABI were both associated with lower verbal memory (Logical Memory) and processing speed (Digit Symbol Coding), with some lesser suggestion of additional associations with non-verbal and working memory measures (Faces, Letter-Number Sequencing; Table 6.4, Table 6.5). Higher cIMT was associated with lower non-verbal memory (Faces), reasoning (Matrix Reasoning) and particularly with lower processing speed as measured by reaction time. For instance, each standard deviation increase in cIMT was associated with a 0.12 standard deviation longer mean Choice Reaction Time latency. Findings were relatively unchanged following multivariable adjustment for conventional vascular risk factors (Table 6.4; Table 6.5), but for *g* lost statistical significance following addition of SIMD as a covariate in the final models (Table 6.4). Additional adjustment for stroke further attenuated the strength of associations.

The subclinical markers therefore appeared to have a relationship with late-life level of cognitive function which was driven by socioeconomic status and by stroke. A contribution of a relatively lower socioeconomic status and/or a prior history of stroke to a lower level of late-life cognitive ability as well as to an increased risk of concurrent presence of subclinical atherosclerosis may be plausible

In contrast to these significant findings for late life cognitive ability assessed as a continuous variable (at least in largely unadjusted analyses), the subclinical markers of macrovascular disease were unrelated to dementia diagnosis. Subjects with dementia had similar NT-proBNP (age- and sex-adjusted geometric means 92.9 pg/ml, 95% CI 54.2, 158.9 versus 81.3 pg/ml, 95% CI 75.8, 87.3, p=0.63), ABI (age- and sex-adjusted mean 0.95, 95% CI 0.85, 1.04 versus 0.98, 95% CI 0.97, 0.99; p=0.45) and cIMT (age- and sex-adjusted mean 0.97 mm, 95% CI 0.88, 1.06 versus 1.00 mm, 95% CI 0.98, 1.01; p=0.58) at baseline compared with participants who were free of dementia.

Table 6.4: Linear regression analyses of associations between measures of subclinical macrovascular disease and late-life cognitive function (*g* and individual cognitive tests) at year 4

	NT-proBNP	Ankle brachial index	Carotid IMT
Logical Memory			
Age, sex adjusted	**-0.09 (0.014)**	**0.11 (0.003)**	-0.05 (0.16)
Multi-adjusted[a]	**-0.09 (0.010)**	**0.11 (0.003)**	-0.05 (0.22)
Faces			
Age, sex adjusted	-0.07 (0.06)	**0.07 (0.041)**	**-0.09 (0.014)**
Multi-adjusted[a]	-0.06 (0.11)	0.06 (0.13)	**-0.08 (0.025)**
Letter Number Sequencing			
Age, sex adjusted	**-0.07 (0.048)**	0.05 (0.14)	-0.01 (0.88)
Multi-adjusted[a]	-0.06 (0.09)	0.05 (0.22)	0.02 (0.70)
Trail-Making			
Age, sex adjusted	0.07 (0.06)	**-0.10 (0.004)**	0.06 (0.09)
Multi-adjusted[a]	0.06 (0.09)	**-0.09 (0.021)**	0.05 (0.15)
Verbal Fluency			
Age, sex adjusted	-0.06 (0.08)	0.02 (0.51)	-0.04 (0.28)
Multi-adjusted[a]	-0.06 (0.09)	0.03 (0.49)	-0.03 (0.47)
Digit Symbol Coding			
Age, sex adjusted	**-0.07 (0.038)**	**0.13 (<0.001)**	-0.05 (0.19)
Multi-adjusted[a]	-0.07 (0.07)	**0.10 (0.006)**	-0.04 (0.31)
Matrix Reasoning			
Age, sex adjusted	-0.04 (0.21)	0.06 (0.09)	**-0.09 (0.010)**
Multi-adjusted[a]	-0.03 (0.42)	0.03 (0.38)	**-0.08 (0.026)**
g			
Age, sex adjusted	**-0.10 (0.005)**	**0.12 (0.001)**	**-0.09 (0.015)**
Multi-adjusted[a]	**-0.09 (0.012)**	**0.10 (0.007)**	**-0.08 (0.043)**
+SIMD[b]	-0.06 (0.07)	0.05 (0.13)	-0.07 (0.07)
+stroke[c]	-0.05 (0.14)	0.04 (0.30)	-0.05 (0.13)

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. NT-proBNP, N-terminal pro-brain natriuretic peptide; Carotid IMT, carotid intima-media thickness; SIMD, Scottish Index of Multiple Deprivation (quintiles). Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test.

[a]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.

[b]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles).

[c]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles), stroke

NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917.

Table 6.5: Linear regression analyses of associations between measures of subclinical macrovascular disease and late-life reaction time at year 4

	NT-proBNP	Ankle brachial index	Carotid IMT
Simple Reaction Time (SRT) mean **Age, sex adjusted**	0.00 (0.92)	-0.06 (0.09)	**0.10 (0.005)**
Multi-adjusted[a]	-0.01 (0.75)	-0.06 (0.11)	**0.09 (0.016)**
Choice Reaction Time (CRT) mean **Age, sex adjusted**	0.04 (0.31)	**-0.08 (0.034)**	**0.12 (0.002)**
Multi-adjusted[a]	0.02 (0.60)	-0.06 (0.11)	**0.10 (0.008)**

Values are standardised β coefficients (p-values). Simple Reaction Time mean is log-transformed. NT-proBNP, N-terminal pro-brain natriuretic peptide; Carotid IMT, carotid intima-media thickness;
[a]adjusted for age, sex, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit. NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917.

6.3 Macrovascular disease and estimated lifetime cognitive change

6.3.1 Symptomatic disease

The relationship between symptomatic macrovascular disease and the estimated lifetime cognitive change between young adulthood and later life was also investigated. For this purpose, multiple linear regression analyses of *g* and scores on the individual cognitive tests was performed on the each of the measures of symptomatic macrovascular disease, with initial adjustment for age, sex and estimated peak pre-morbid ability (MHVS). In a subsequent step, conventional vascular risk factors were added as covariates into the model, before, for analyses of *g*, SIMD representing socioeconomic status was additionally controlled for. The results from analyses of *g* and a majority of individual cognitive tests are shown in Table 6.6 and for reaction time measures are shown in Table 6.7. All symptomatic disease groups were relatively unrelated to lifetime cognitive change; significance at the 5% level was typically restricted to a small number of individual cognitive tests rather than *g* and effect sizes were relatively small throughout. An exception to this was stroke, which was consistently associated with a steeper decline between peak pre-morbid ability (which was presumably the level of ability prior to stroke) and post-stroke late-life cognitive function, with effect sizes which appeared somewhat reduced compared with the analyses of year 4 level of cognitive function (Table 6.2; Table 6.3). For instance, a history of stroke was associated with a 0.12 standard deviation lower *g* with age, sex and estimated peak pre-morbid ability held constant. Overall, the finding shows that even several (at least four) years following the event, potential post-stroke cognitive recovery had not achieved a level of cognitive function which would be expected given an individual's pre-morbid ability in young adulthood. Multivariable adjustment for conventional vascular risk factors or for socioeconomic status did not appear to change these findings.

Table 6.6: Linear regression analyses of associations between symptomatic macrovascular disease and late-life cognitive function (*g* and individual cognitive tests) at year 4 adjusted for estimated peak pre-morbid ability (MHVS)

	Stroke (n=44)		TIA (n=27)		MI (n=111)		Angina (n=222)		PAD (n=53)	
	Vs. no stroke	**Vs. no symptomatic MVD**	**Vs. no TIA**	**Vs. no symptomatic MVD**	**Vs. no MI**	**Vs. no symptomatic MVD**	**Vs. no angina**	**Vs. no symptomatic MVD**	**Vs. no PAD**	**Vs. no symptomatic MVD**
Logical Memory										
Initial model[a]	-0.04 (0.17)	-0.06 (0.13)	0.03 (0.29)	0.04 (0.32)	0.00 (0.95)	-0.01 (0.87)	-0.02 (0.65)	-0.02 (0.55)	-0.03 (0.33)	-0.04 (0.25)
Multi-adjusted[b]	-0.05 (0.15)	-0.06 (0.10)	0.03 (0.31)	0.03 (0.40)	-0.01 (0.81)	-0.02 (0.54)	-0.03 (0.43)	-0.03 (0.32)	-0.04 (0.27)	-0.05 (0.18)
Faces										
Initial model[a]	-0.04 (0.28)	-0.05 (0.21)	-0.04 (0.28)	-0.05 (0.21)	-0.02 (0.58)	-0.03 (0.37)	0.00 (0.95)	-0.02 (0.66)	-0.06 (0.08)	-0.07 (0.09)
Multi-adjusted[b]	-0.03 (0.41)	-0.04 (0.29)	-0.04 (0.29)	-0.05 (0.20)	-0.02 (0.60)	-0.04 (0.33)	0.00 (0.91)	-0.02 (0.59)	-0.06 (0.09)	-0.08 (0.06)
Letter Number										
Initial model[a]	-0.05 (0.14)	**-0.08 (0.032)**	-0.01 (0.74)	-0.03 (0.44)	-0.04 (0.18)	-0.06 (0.11)	-0.01 (0.80)	-0.02 (0.53)	-0.03 (0.42)	-0.04 (0.32)
Multi-adjusted[b]	-0.05 (0.15)	-0.07 (0.10)	-0.01 (0.69)	-0.02 (0.62)	-0.05 (0.15)	-0.05 (0.18)	-0.02 (0.66)	-0.02 (0.50)	-0.03 (0.38)	-0.04 (0.33)
Trail-Making										
Initial model[a]	**0.12 (<0.001)**	**0.16 (<0.001)**	0.00 (0.98)	0.01 (0.88)	0.04 (0.27)	0.04 (0.25)	0.00 (0.97)	0.01 (0.70)	0.03 (0.29)	0.05 (0.22)
Multi-adjusted[b]	**0.12 (<0.001)**	**0.14 (<0.001)**	0.00 (0.97)	0.00 (0.95)	0.04 (0.26)	0.05 (0.20)	0.00 (0.96)	0.02 (0.62)	0.03 (0.32)	0.04 (0.31)
Verbal Fluency										
Initial model[a]	**-0.08 (0.012)**	**-0.10 (0.013)**	0.02 (0.64)	0.02 (0.71)	0.01 (0.81)	0.00 (0.97)	0.03 (0.35)	0.02 (0.67)	-0.03 (0.36)	-0.03 (0.39)
Multi-adjusted[b]	**-0.08 (0.018)**	**-0.09 (0.015)**	0.01 (0.75)	0.01 (0.82)	0.00 (0.93)	-0.01 (0.73)	0.02 (0.51)	0.00 (0.94)	-0.04 (0.23)	-0.04 (0.26)
Digit Symbol Coding **Initial model**[a]	**-0.13 (<0.001)**	**-0.18 (<0.001)**	0.00 (0.97)	0.00 (0.92)	-0.05 (0.11)	**-0.06 (0.09)**	**-0.07 (0.034**)	**-0.07 (0.030)**	0.00 (0.89)	-0.22 (0.56)
Multi-adjusted[b]	**-0.12 (<0.001)**	**-0.14 (<0.001)**	0.01 (0.89)	0.00 (0.97)	-005 (0.13)	-0.06 (0.10)	-0.07 (0.025)	-0.08 (0.020)	0.02 (0.64)	0.01 (0.88)
Matrix Reasoning										
Initial model[a]	-0.03 (0.30)	-0.04 (0.29)	**-0.08 (0.009)**	**-0.10 (0.009)**	**-0.08 (0.015)**	**-0.08 (0.030)**	0.01 (0.80)	0.00 (0.99)	0.01 (0.66)	0.01 (0.78)
Multi-adjusted[b]	-0.03 (0.36)	-0.03 (0.38)	**-0.08 (0.009)**	**-0.09 (0.012)**	**-0.08 (0.017)**	**-0.07 (0.052)**	0.01 (0.77)	0.01 (0.89)	0.02 (0.57)	0.02 (0.61)
g										
Initial model[a]	**-0.12 (<0.001)**	**-0.15 (<0.001)**	-0.02 (0.59)	-0.03 (0.40)	-0.05 (0.11)	-0.06 (0.051)	-0.01 (0.75)	-0.03 (0.31)	-0.03 (0.27)	-0.05 (0.15)
Multi-adjusted[b]	**-0.12 (<0.001)**	**-0.14 (<0.001)**	-0.02 (0.58)	-0.03 (0.44)	-0.05 (0.10)	-0.06 (0.054)	-0.02 (0.63)	-0.03 (0.28)	-0.03 (0.27)	-0.04 (0.20)
+SIMD[c]	**-0.11 (<0.001)**	**-0.13 (<0.001)**	-0.01 (0.75)	-0.02 (0.63)	-0.04 (0.19)	-0.05 (0.12)	0.00 (0.95)	-0.02 (0.60)	-0.03 (0.33)	-0.04 (0.28)

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. For each independent variable, comparisons are made with the respective remaining sample and with participants free of any symptomatic MVD (n=538). MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease; SIMD, Scottish Index of Multiple Deprivation (quintiles); Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; MHVS, Mill-Hill Vocabulary Test; Letter Number, Letter Number Sequencing.
[a]adjusted for age, sex, baseline MHVS.
[b]adjusted models adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.
[c]adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles).

Table 6.7: Linear regression analyses of associations between symptomatic macrovascular disease and late-life reaction time at year 4 adjusted for estimated peak pre-morbid ability (MHVS)

	Simple Reaction Time (SRT) mean		**Choice Reaction Time (CRT) mean**	
	Initial model[a]	**Multi-adjusted[b]**	**Initial model[a]**	**Multi-adjusted[b]**
Stroke **vs. no stroke**	**0.08 (0.020)**	**0.08 (0.020)**	0.03 (0.35)	0.03 (0.35)
vs. no symptomatic MVD	**0.09 (0.039)**	**0.09 (0.039)**	0.04 (0.36)	0.04 (0.35)
TIA **vs. no TIA**	0.01 (0.76)	0.01 (0.72)	0.01 (0.88)	0.01 (0.81)
vs. no symptomatic MVD	0.01 (0.74)	0.02 (0.71)	0.01 (0.83)	0.01 (0.74)
MI **vs. no MI**	-0.03 (0.34)	-0.04 (0.33)	-0.04 (0.33)	-0.04 (0.34)
vs. no symptomatic MVD	-0.02 (0.60)	-0.02 (0.55)	-0.03 (0.55)	-0.02 (0.66
Angina **vs. no angina**	0.00 (0.95)	0.00 (0.95)	0.04 (0.30)	0.04 (0.30)
vs. no symptomatic MVD	0.01 (0.85)	0.00 (0.97)	0.04 (0.36)	0.03 (0.41)
PAD **vs. no PAD**	-0.01 (0.86)	-0.01 (0.85)	0.07 (0.07)	0.07 (0.07)
vs. no symptomatic MVD	-0.01 (0.88)	0.00 (0.92)	0.07 (0.08)	0.08 (0.052)

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up reaction time scores. Simple Reaction Time mean is log-transformed. MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease.
[a]adjusted for age, sex, baseline MHVS. [b]adjusted models adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.
Stroke n=44; TIA, n=27; MI, n=111; angina n=22; PAD n=53; no symptomatic MVD n=538.

6.3.2 Subclinical disease

Multiple linear regression analyses of *g* and of scores on the individual cognitive tests at year 4 on ABI, cIMT and NT-proBNP were performed. Each initial model controlled for age, sex and baseline MHVS (estimating peak pre-morbid ability) to represent associations of the respective predictor with estimated lifetime cognitive change. A second model additionally controlled for conventional vascular risk factors. For *g*, two further models controlled for SIMD and for stroke in separate steps in order to show potential confounding of overall findings by socioeconomic status or by stroke. Table 6.8 shows the results for *g* and a majority of individual cognitive tests and Table 6.9 shows the results for the reaction time measures.

Increased severity of subclinical macrovascular disease as measured by ABI, cIMT and NT-proBNP was associated with steeper estimated lifetime decline in *g*, with the specific cognitive domains as well as relative effect sizes mirroring those which had been identified as significant for the level of cognitive function at year 4 (Table 6.4; Table 6.5). The finding shows that late-life atherosclerosis was linked to an accelerated cognitive decline between younger adulthood and older age. Carotid IMT stood out as the only predictor of estimated lifetime decline in reaction time (Table 6.5). The association had the relatively largest effect size amongst all analyses of the subclinical markers and the estimated lifetime change in the various cognitive tests. Each standard deviation increase in cIMT was associated with a 0.12 standard deviation increase in mean Choice Reaction Time with age, sex and estimated peak pre-morbid ability (MHVS) held constant. Again, the results were largely unchanged when vascular risk factors were controlled for. Associations with *g* remained statistically significant for NT-proBNP and cIMT, but not for ABI, following adjustment for SIMD; additional adjustment for stroke then rendered all findings except that for cIMT statistically non-significant (Table 6.8).- Overall, the association of late-life atherosclerosis of the carotid artery but not of the periphery or of the heart appeared to be related to the decline between estimated peak ability in young adulthood and later life, independent of socioeconomic status or stroke.

Table 6.8: Linear regression analyses of associations between subclinical macrovascular disease and late-life cognitive function at year 4 (*g* and individual cognitive tests) adjusted for estimated peak pre-morbid ability (MHVS)

	NT-proBNP	Ankle brachial index	Carotid IMT
Logical Memory			
Initial model[a]	**-0.07 (0.033)**	**0.08 (0.017)**	-0.06 (0.08)
Multi-adjusted[b]	**-0.08 (0.014)**	**0.09 (0.007)**	-0.06 (0.08)
Faces			
Initial model[a]	-0.05 (0.13)	0.05 (0.14)	**-0.09 (0.008)**
Multi-adjusted[b]	-0.05 (0.17)	0.04 (0.24)	**-0.09 (0.011)**
Letter Number Sequencing			
Initial model[a]	-0.05 (0.12)	0.02 (0.56)	-0.07 (0.71)
Multi-adjusted[b]	-0.05 (0.15)	0.02 (0.58)	0.00 (0.97)
Trail-Making			
Initial model[a]	0.05 (0.14)	**-0.07 (0.030)**	0.06 (0.06)
Multi-adjusted[b]	0.05 (0.16)	-0.06 (0.07)	0.06 (0.08)
Verbal Fluency			
Initial model[a]	-0.05 (0.17)	-0.01 (0.81)	-0.05 (0.18)
Multi-adjusted[b]	-0.05 (0.14)	0.00 (0.92)	-0.04 (0.26)
Digit Symbol Coding			
Initial model[a]	-0.05 (0.11)	**0.11 (0.001)**	-0.05 (0.13)
Multi-adjusted[b]	-0.05 (0.14)	**0.08 (0.014)**	-0.05 (0.17)
Matrix Reasoning			
Initial model[a]	-0.03 (0.38)	0.02 (0.44)	**-0.10 (0.002)**
Multi-adjusted[b]	-0.02 (0.52)	0.01 (0.81)	**-0.10 (0.005)**
g			
Initial model[a]	**-0.07 (0.013)**	**0.08 (0.007)**	**-0.10 (0.002)**
Multi-adjusted[b]	**-0.07 (0.017)**	**0.07 (0.020)**	**-0.09 (0.005)**
+SIMD[c]	**-0.06 (0.038)**	0.04 (0.20)	**-0.08 (0.006)**
+stroke[d]	-0.05 (0.08)	0.02 (0.42)	**-0.07 (0.014)**

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. NT-proBNP, N-terminal pro-brain natriuretic peptide; Carotid IMT, carotid intima-media thickness; SIMD, Scottish Index of Multiple Deprivation (quintiles); Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test.

[a]adjusted for age, sex, baseline MHVS.

[b]adjusted models adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.

[c]adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles).

[d]adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, SIMD (quintiles), stroke

NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917.

Table 6.9: Linear regression analyses of associations between subclinical macrovascular disease and late-life reaction time at year 4 adjusted for estimated peak pre-morbid ability (MHVS)

	NT-proBNP	Ankle brachial index	Carotid IMT
Simple Reaction Time mean			
Initial model[a]	-0.01 (0.72)	-0.05 (0.13)	**0.11 (0.004)**
Multi-adjusted[b]	-0.03 (0.47)	-0.05 (0.16)	**0.09 (0.012)**
Choice Reaction Time mean			
Initial model[a]	0.03 (0.47)	-0.06 (0.13)	**0.12 (0.001)**
Multi-adjusted[b]	0.01 (0.78)	-0.04 (0.29)	**0.11 (0.005)**

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up reaction time scores. Simple Reaction Time mean is log-transformed. NT-proBNP, N-terminal pro-brain natriuretic peptide; carotid IMT, carotid intima-media thickness.
[a]adjusted for age, sex, baseline MHVS.
[b]adjusted for age, sex, baseline MHVS, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.
NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917.

6.4 Macrovascular disease and four-year cognitive change

6.4.1 Symptomatic disease

Multiple linear regression models of follow-up *g* and scores on the seven cognitive tests contributing to *g* on each of the macrovascular predictors with adjustment for the respective baseline scores tested associations between symptomatic macrovascular disease and the change in cognitive function between baseline and year 4. Because participants' change in cognitive function between baseline and year 4 follow-up is the main outcome of interest in this thesis, unadjusted associations of each of the macrovascular disease categories (compared with all remaining participants) with the four-year change in *g* are illustrated in Figure 6.2 to 6.6. Note that comparison of observation between graphs is not possible due to varying scales used on the Y-axis.

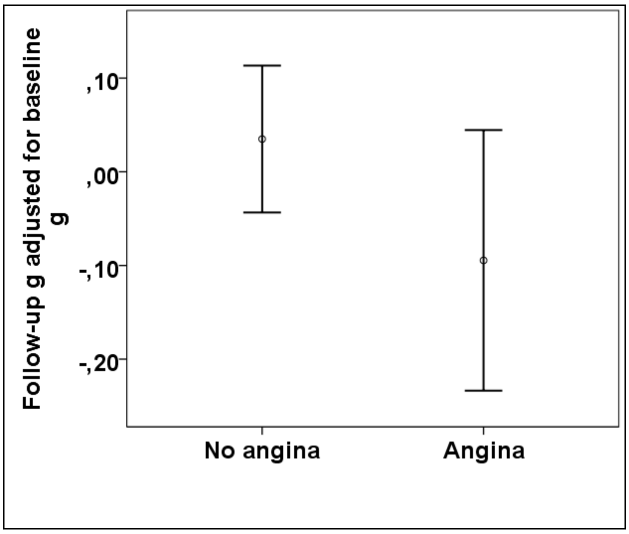
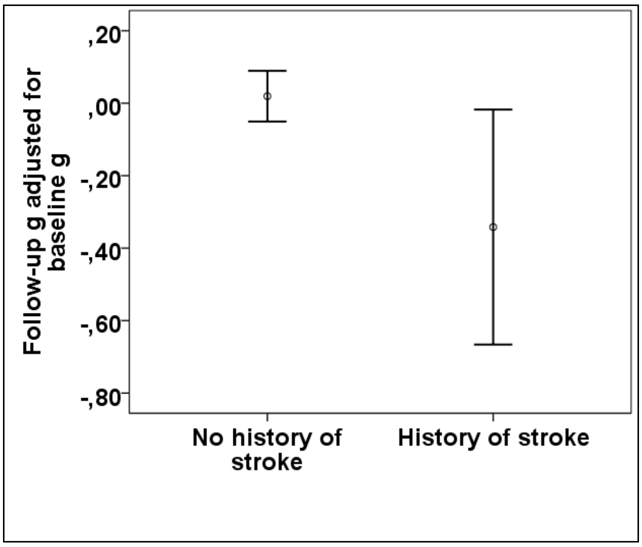
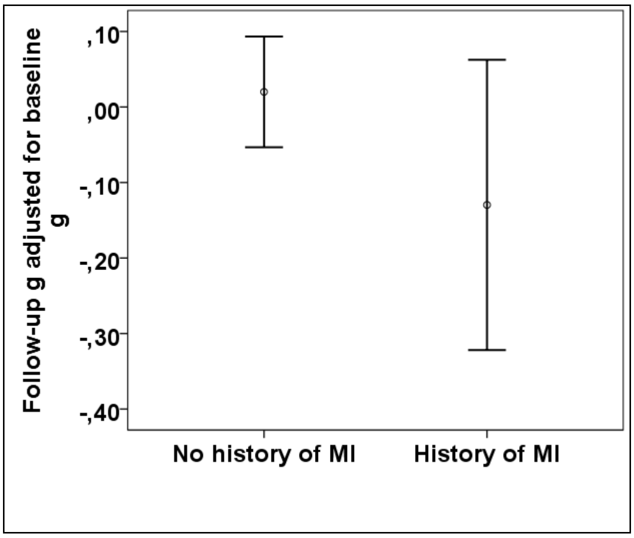


Figure 6.2: Mean follow-up *g* adjusted for baseline *g* in subjects with angina and those without angina at baseline (error bars show 95% CI)



<u>Figure 6.3: Mean follow-up *g* adjusted for baseline *g* in subjects with a baseline history of stroke and those without a baseline history of stroke (error bars show 95% CI)</u>



<u>Figure 6.4: Mean follow-up *g* adjusted for baseline *g* in subjects with a history of MI and those without a history of MI at baseline (error bars show 95% CI)</u>

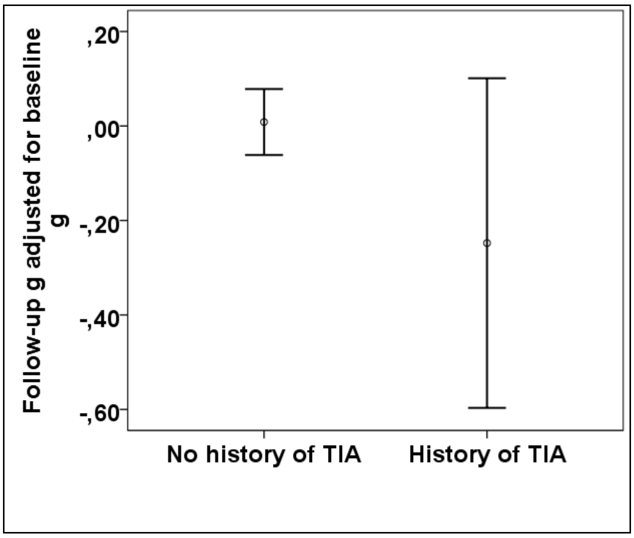


Figure 6.5: Mean follow-up *g* adjusted for baseline *g* in subjects with a history of TIA and those without a history of TIA at baseline (error bars show 95% CI)

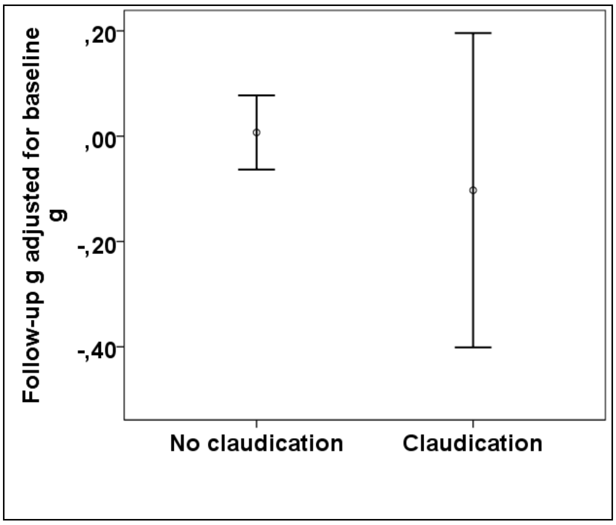


Figure 6.6: Mean follow-up *g* adjusted for baseline *g* in subjects with claudication and those without claudication at baseline (error bars show 95% CI)

In linear regression models exploring these observations, an initial model controlled for age and sex, before conventional vascular risk factors were entered as covariates. Baseline MHVS was controlled for in a next model with the aim to show the independence of findings from individual differences in peak pre-morbid cognitive ability. For *g*, a final model then additionally controlled for SIMD measuring participants' socioeconomic status. Analyses compared the symptomatic disease variables with the respective remaining sample and with participants free of any macrovascular disease (n=538). Results are shown in Table 6.10.

Similar to the analyses of estimated lifetime cognitive change presented in the previous section (Table 6.6, Table 6.7), stroke was the only symptomatic disease group consistently associated with four-year cognitive change in later life. The remaining symptomatic disease groups were only occasionally significantly related to declines on individual cognitive tests and with relatively limited effect sizes; findings for PAD were consistently non-significant. In addition to *g*, stroke at baseline appeared to accelerate particularly the decline in processing speed (Digit Symbol Coding) and executive function (Trail-Making Test-B). A majority of the significant findings for stroke were not attenuated following adjustment for vascular risk factors.

Although data on the specific time at which the cerebral infarction occurred in subjects identified to have a history of stroke, it appears to be likely that it had occurred months or years prior to the baseline clinic, considering that clinic attendance was required for inclusion in the study. The finding on first inspection therefore suggests that cerebral infarction has lasting effects on subsequent trajectories of cognitive ageing. The effect sizes, however, were notably smaller compared with the associations of stroke with estimated lifetime cognitive decline. For instance, stroke was associated with a 0.07 standard deviation lower score on year 4 *g* with age, sex and baseline *g* held constant (Table 6.10), whereas it was associated with a 0.12 standard deviation lower *g* with age, sex and MHVS held constant (Table 6.6). Some confounding by individual differences in pre-morbid ability and by socioeconomic status was also indicated by an attenuation of the results following adjustment for MHVS and for SIMD, overall demonstrating that lasting effects of stroke on post-stroke cognitive change which were independent of these factors were relatively limited.

Table 6.10: Linear regression analyses of associations between symptomatic macrovascular disease and four-year cognitive change (*g* and individual cognitive tests)

	Stroke* (n=44)		TIA* (n=27)		MI* (n=111)		Angina* (n=222)		PAD* (n=53)	
	Vs. no stroke	**Vs. no symptomatic MVD**	**Vs. no TIA**	**Vs. no symptomatic MVD**	**Vs. no MI**	**Vs. no symptomatic MVD**	**Vs. no angina**	**Vs. no symptomatic MVD**	**Vs. no PAD**	**Vs. no symptomatic MVD**
Logical Memory										
Initial model[a]	-0.03 (0.30)	-0.06 (0.09)	0.01 (0.86)	0.00 (0.90)	-0.03 (0.23)	-0.06 (0.08)	**-0.06 (0.039)**	**-0.07 (0.017)**	-0.05 (0.08)	**-0.07 (0.032)**
Multi-adjusted[b]	-0.03 (0.28)	-0.06 (0.07)	0.01 (0.84)	-0.01 (0.84)	-0.03 (0.24)	-0.06 (0.06)	**-0.06 (0.043)**	**-0.07 (0.014)**	-0.05 (0.10)	**-0.07 (0.024)**
+MHVS[c]	-0.02 (0.42)	-0.04 (0.21)	0.02 (0.41)	0.02 (0.56)	-0.02 (0.47)	-0.03 (0.28)	-0.02 (0.40)	-0.03 (0.25)	-0.04 (0.18)	-0.05 (0.10)
Faces										
Initial model[a]	-0.02 (0.59)	-0.03 (0.44)	-0.04 (0.21)	-0.05 (0.13)	0.00 (0.88)	-0.02 (0.62)	-0.02 (0.41)	-0.04 (0.22)	-0.05 (0.11)	-0.05 (0.10)
Multi-adjusted[b]	-0.01 (0.76)	-0.02 (0.60)	-0.04 (0.21)	-0.05 (0.13)	0.01 (0.84)	-0.02 (0.60)	-0.02 (0.43)	-0.04 (0.23)	-0.04 (0.13)	-0.06 (0.07)
+MHVS[c]	0.00 (0.98)	0.00 (0.91)	-0.03 (0.25)	-0.04 (0.20)	0.01 (0.69)	0.00 (0.89)	0.00 (0.93)	-0.01 (0.68)	-0.04 (0.13)	-0.04 (0.13)
Letter Number										
Initial model[a]	-0.04 (0.25)	**-0.07 (0.048)**	-0.01 (0.70)	-0.04 (0.32)	**-0.07 (0.031)**	**-0.08 (0.013)**	-0.05 (0.11)	**-0.06 (0.046)**	-0.04 (0.23)	-0.06 (0.11)
Multi-adjusted[b]	-0.03 (0.29)	-0.06 (0.12)	-0.01 (0.65)	-0.03 (0.46)	-0.07 (0.031)	**-0.08 (0029)**	-0.05 (0.12)	-0.06 (0.07)	-0.04 (0.23)	-0.06 (0.13)
+MHVS[c]	-0.03 (0.35)	-0.04 (0.26)	-0.01 (0.77)	-0.01 (0.71)	-0.05 (0.09)	-0.05 (0.16)	-0.01 (0.66)	-0.02 (0.53)	-0.01 (0.63)	-0.02 (0.58)
Trail-Making										
Initial model[a]	**0.06 (0.030)**	**0.11 (<0.001)**	0.01 (0.68)	0.03 (0.43)	0.03 (0.19)	0.05 (0.10)	0.04 (0.13)	0.05 (0.09)	0.01 (0.68)	0.05 (0.14)
Multi-adjusted[b]	**0.06 (0.032)**	**0.08 (0.015)**	0.01 (0.74)	0.02 (0.58)	0.03 (0.24)	0.05 (0.10)	0.03 (0.19)	0.05 (0.10)	0.01 (0.85)	0.02 (0.62)
+MHVS[c]	**0.05 (0.037)**	**0.07 (0.034)**	0.00 (0.96)	0.00 (0.98)	0.02 (0.37)	0.03 (0.29)	0.01 (0.68)	0.02 (0.48)	0.00 (0.91)	0.01 (0.83)
Verbal Fluency										
Initial model[a]	-0.04 (0.07)	**-0.05 (0.038)**	0.00 (0.97)	-0.01 (0.76)	-0.01 (0.81)	-0.01 (0.66)	-0.03 (0.15)	-0.03 (0.16)	0.01 (0.70)	-0.02 (0.50)
Multi-adjusted[b]	-0.04 (0.07)	-0.05 (0.031)	0.00 (0.91)	-0.01 (0.64)	-0.01 (0.68)	-0.02 (0.42)	-0.03 (0.14)	-0.04 (0.08)	-0.01 (0.62)	-0.02 (0.38)
+MHVS[c]	-0.03 (0.10)	-0.05 (0.07)	0.01 (0.78)	0.00 (0.93)	-0.01 (0.82)	-0.01 (0.68)	-0.02 (0.42)	-0.02 (0.30)	-0.01 (0.55)	-0.02 (0.43)
Digit Symbol Coding										
Initial model[a]	**-0.06 (0.011)**	**-0.09 (0.001)**	-0.01 (0.68)	-0.01 (0.74)	-0.02 (0.53)	-0.02 (0.46)	**-0.05 (0.022)**	**-0.05 (0.036)**	0.02 (0.37)	0.00 (0.91)
Multi-adjusted[b]	**-0.06 (0.012)**	**-0.07 (0.014)**	-0.01 (0.72)	-0.01 (0.63)	-0.02 (0.50)	-0.03 (0.35)	**-0.06 (0.020)**	**-0.06 (0.026)**	0.03 (0.25)	0.02 (0.43)
+MHVS[c]	**-0.06 (0.015)**	**-0.06 (0.028)**	0.01 (0.83)	0.01 (0.84)	-0.01 (0.72)	-0.01 (0.70)	-0.04 (0.12)	-0.04 (0.17)	0.03 (0.19)	0.03 (0.26)
Matrix Reasoning										
Initial model[a]	-0.03 (0.20)	-0.03 (0.36)	**-0.08 (0.002)**	**-0.10 (0.002)**	-0.04 (0.10)	-0.04 (0.17)	0.00 (0.93)	-0.01 (0.76)	0.01 (0.70)	0.02 (0.62)

Multi-adjusted[b]	-0.03 (0.19)	-0.04 (0.23)	**-0.08 (0.002)**	**-0.10 (0.002)**	-0.05 (0.08)	-0.04 (0.19)	0.00 (0.93)	-0.01 (0.81)	0.01 (0.66)	0.01 (0.74)
+MHVS[c]	-0.02 (0.35)	-0.02 (0.52)	**-0.07 (0.005)**	**-0.08 (0.009)**	-0.05 (0.07)	-0.03 (0.30)	0.03 (0.36)	0.02 (0.40)	0.02 (0.50)	0.03 (0.43)
g **Initial model**[a]	**-0.07 (0.036)**	**-0.10 (0.014)**	-0.05 (0.18)	-0.07 (0.09)	-0.04 (0.29)	-0.06 (0.13)	-0.04 (0.24)	-0.06 (0.08)	-0.02 (0.57)	-0.04 (0.29)
Multi-adjusted[b]	**-0.07 (0.037)**	**-0.10 (0.016)**	-0.05 (0.18)	-0.07 (0.10)	-0.04 (0.28)	-0.06 (0.16)	-0.04 (0.29)	-0.06 (0.14)	-0.01 (0.70)	-0.03 (0.45)
+MHVS[c]	-0.06 (0.07)	-0.08 (0.07)	-0.03 (0.39)	-0.04 (0.33)	-0.03 (0.44)	-0.03 (0.47)	-0.01 (0.74)	-0.02 (0.54)	0.01 (0.83)	0.00 (0.97)
+SIMD[d]	-0.06 (0.10)	-0.07 (0.11)	-0.02 (0.49)	-0.03 (0.45)	-0.02 (0.62)	-0.02 (0.66)	0.00 (0.94)	-0.01 (0.82)	0.01 (0.76)	0.01 (0.85)

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. For each independent variable, comparisons are made with the respective remaining sample and with participants free of any symptomatic MVD (n=538). MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease; MHVS, Mill-Hill Vocabulary Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; Letter Number, Letter Number Sequencing.

[a]adjusted for age, sex, baseline scores.

[b]adjusted models adjusted for age, sex, baseline scores, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.

[c]adjusted for age, sex, baseline scores, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, baseline MHVS.

[d]adjusted for age, sex, baseline *g*, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, baseline MHVS, SIMD (quintiles).

6.4.2 Subclinical disease

The analyses of four-year cognitive change for the subclinical markers of macrovascular disease were also performed using multiple regression models of follow-up *g* and scores on the individual cognitive tests contributing to *g* on ABI, NT-proBNP and cIMT. Unadjusted associations are illustrated in Figures 6.7 to 6.9.

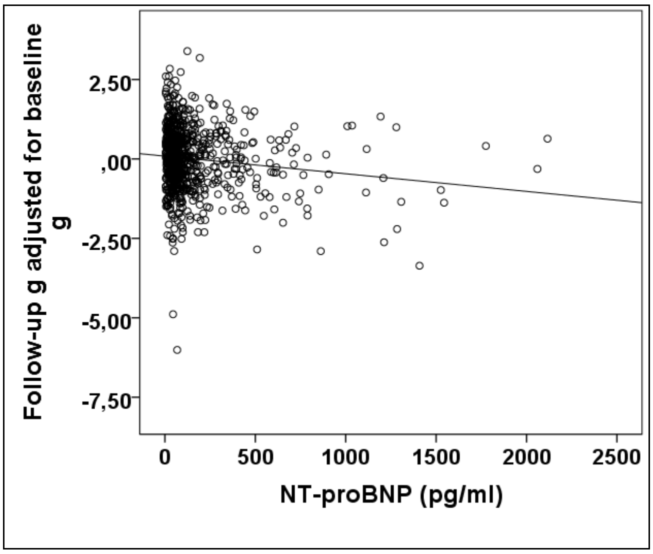


Figure 6.7: Scatterplot illustrating association of baseline NT-proBNP (prior to log-transformation) with four-year change in *g*

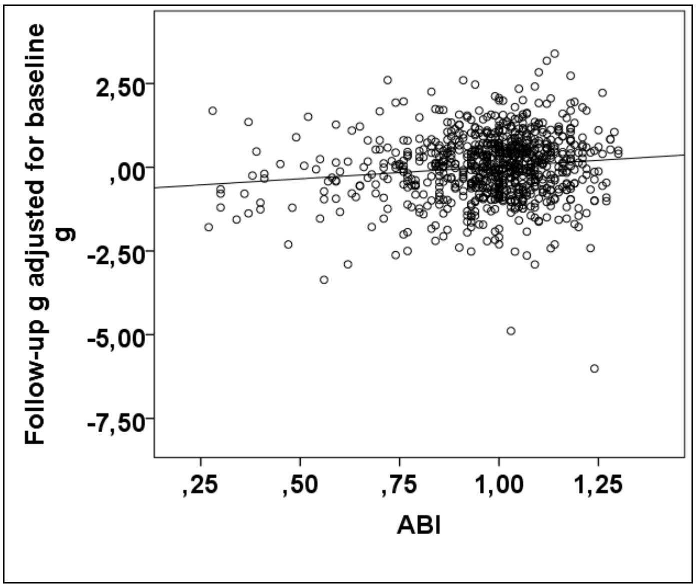
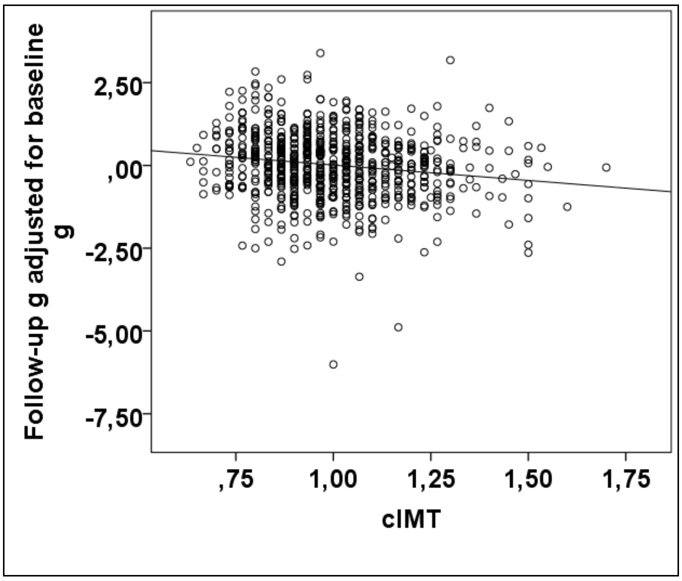


Figure 6.8: Scatterplot illustrating association of baseline ABI with four-year change in *g*



<u>Figure 6.9: Scatterplot illustrating association of cIMT measured at year 1 with change in *g* between baseline and year 4</u>

In analyses exploring the observation of associations based on graphical presentation, the respective initial linear regression model controlled for age and sex; conventional vascular risk factors and estimated peak pre-morbid ability (baseline MHVS) were subsequently controlled for in two separate steps, with additional adjustment for socioeconomic status (SIMD) and stroke in separate steps for the analyses of *g* only. Results of these analyses are shown in Table 6.11. Increased severity of subclinical macrovascular disease (as measured by ABI, NT-proBNP and cIMT) predicted an accelerated decline in *g* between baseline and year 4. Effect sizes were similar for ABI and NT-proBNP, and relatively largest for cIMT. Higher cIMT was associated with steeper decline on a majority of cognitive tests, some of which (verbal memory, processing speed) were shared with ABI and NT-proBNP. Additionally, higher cIMT was also linked to steeper decline in non-verbal memory (Faces) and reasoning (Matrix Reasoning). Higher NT-proBNP was further associated with steeper decline in working memory (Letter-Number Sequencing) and in executive function measured by verbal fluency - domains which it had only marginally been linked to in analyses of year 4 level of cognitive function (Table 6.4) and of estimated lifetime decline (Table 6.8).

Compared with the findings for estimated lifetime cognitive change (Table 6.8; Table 6.9), the strength of associations was increased particularly for NT-proBNP and cIMT. For instance, each standard deviation increase in baseline cIMT was associated with a 0.15 lower follow-up *g* with age, sex and baseline *g* held constant. All associations survived multivariable adjustment for vascular risk factors and were largely unaffected by adjustment for MHVS or, for analyses of *g*, by additional adjustment for SIMD or for stroke (Table 6.11) This suggests that individuals with greater atherosclerotic burden at baseline experienced a steeper subsequent cognitive decline independent of potential confounding by a prior history of stroke, by individual differences in peak pre-morbid ability or by socioeconomic status.

Table 6.11: Linear regression analyses of associations between subclinical macrovascular disease and four-year cognitive change (*g* and individual cognitive tests)

	NT-proBNP	Ankle brachial index	Carotid IMT
Logical Memory			
Initial model[a]	**-0.07 (0.013)**	**0.08 (0.006)**	**-0.06 (0.030)**
Multi-adjusted[b]	**-0.06 (0.028)**	**0.07 (0.021)**	**-0.05 (0.08)**
+MHVS[c]	**-0.06 (0.030)**	**0.06 (0.027)**	**-0.06 (0.040)**
Faces			
Initial model[a]	-0.04 (0.15)	0.04 (0.12)	**-0.08 (0.007)**
Multi-adjusted[b]	-0.04 (0.23)	0.03 (0.25)	**-0.08 (0.011)**
+MHVS[c]	-0.03 (0.27)	0.03 (0.35)	**-0.08 (0.006)**
Letter Number Sequencing			
Initial model[a]	**-0.08 (0.014)**	0.05 (0.13)	-0.02 (0.46)
Multi-adjusted[b]	**-0.07 (0.027)**	0.04 (0.23)	-0.01 (0.83)
+MHVS[c]	-0.06 (0.06)	0.02 (0.46)	-0.01 (0.69)
Trail-Making			
Initial model[a]	0.04 (0.11)	**-0.06 (0.012)**	0.05 (0.08)
Multi-adjusted[b]	0.03 (0.23)	**-0.06 (0.037)**	0.04 (0.14)
+MHVS[c]	0.03 (0.32)	-0.05 (0.06)	0.05 (0.10)
Verbal Fluency			
Initial model[a]	**-0.07 (0.001)**	0.03 (0.12)	-0.02 (0.50)
Multi-adjusted[b]	**-0.07 (0.001)**	0.03 (0.16)	-0.01 (0.78)
+MHVS[c]	**-0.07 (0.001)**	0.02 (0.28)	-0.01 (0.72)
Digit Symbol Coding			
Initial model[a]	**-0.05 (0.053)**	**0.07 (0.004)**	**-0.08 (0.002)**
Multi-adjusted[b]	**-0.04 (0.10)**	**0.05 (0.038)**	**-0.07 (0.006)**
+MHVS[c]	-0.04 (0.14)	**0.05 (0.043)**	**-0.07 (0.005)**
Matrix Reasoning			
Initial Model[a]	-0.01 (0.65)	0.02 (0.54)	**-0.08 (0.003)**

Multi-adjusted[b]	-0.01 (0.79)	0.01 (0.81)	**-0.08 (0.005)**
+MHVS[c]	-0.01 (0.79)	0.00 (0.94)	**-0.09 (0.002)**
g Initial model[a]	**-0.12 (0.001)**	**0.12 (<0.001)**	**-0.15 (<0.001)**
Multi-adjusted[b]	**-0.10 (0.005)**	**0.10 (0.005)**	**-0.12 (0.001)**
+MHVS[c]	**-0.10 (0.008)**	**0.09 (0.018)**	**-0.13 (0.001)**
+SIMD[d]	**-0.09 (0.016)**	**0.07 (0.046)**	**-0.13 (0.001)**
+stroke[e]	**-0.08 (0.022)**	0.06 (0.06)	**-0.12 (0.001)**

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making Test-B is log-transformed. NT-proBNP, N-terminal pro-brain natriuretic peptide; Carotid IMT, carotid intima-media thickness; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; MHVS, Mill-Hill Vocabulary Scale.
[a]adjusted for age, sex, baseline scores.
[b]adjusted models adjusted for age, sex, baseline scores, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit.
[c]adjusted for age, sex, baseline scores, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, baseline MHVS.
[d]adjusted for age, sex, baseline *g*, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, baseline MHVS, SIMD (quintiles)
[e]adjusted for age, sex, baseline *g*, total cholesterol, smoking (current/ex/never), systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, baseline MHVS, SIMD (quintiles), stroke
NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917.

6.5 Summary

To summarise, effect sizes were relatively small throughout the statistically significant findings presented in this chapter. A lower peak pre-morbid ability in young adulthood (which was estimated using a test of vocabulary) was associated with an increased risk of symptomatic and subclinical macrovascular disease at the baseline assessment of the ET2DS, although individual differences in socioeconomic status appeared to play a confounding role in this association.

All of the baseline macrovascular predictors (except TIA) were associated with a lower level of cognitive function at year 4, with significant findings most frequently observed for measures of processing speed and the memory domain.

None of the associations between symptomatic disease manifesting distant from the brain and cognitive function at year 4 survived the adjustment for an estimate of peak pre-morbid ability. These predictors were also overall unrelated to late-life cognitive trajectories. In contrast, a history of stroke stood out as associated with steeper estimated lifetime decline independently of socioeconomic status. Yet, the finding of stroke associations with a steeper four-year decline appeared to be partly driven by estimated peak pre-morbid ability and by socioeconomic status. It therefore appears that the cerebrovascular accident may have relatively more severely affected cognitive function between pre-stroke to post-stroke estimations/assessments irrespective of the participant's socioeconomic status, with a relatively weaker role in the determination of post-stroke cognitive change. It may be the case that participants with a relatively lower peak pre-morbid ability in young adulthood and those with a relatively lower socioeconomic status were at increased risk of later stroke and also experienced an accelerated late-life cognitive decline relative to the remaining sample.

Although the comparison of effect sizes between the symptomatic (categorical) and subclinical (continuous) disease variables is not informative, the overall patterns of findings may be compared between the two groups of predictors. Contrasting the results for stroke, associations of the three subclinical markers of macrovascular disease were, relatively independently of socioeconomic status, overall stronger for analyses of four-year cognitive change than of estimated lifetime change. Moreover, only the latter association was confounded by stroke, further demonstrating that the finding of associations of the subclinical disease markers with four-year cognitive change is relatively stronger and more informative with respect to an individual patient's risk of cognitive impairment. A greater atherosclerotic burden of the body at baseline preceded an accelerated cognitive decline and did so independent of all potential confounders considered here, including stroke and socioeconomic status.

Chapter 7: Results III: Severe hypoglycaemia and cognition

7.1 Overview and statistical methods

A number of investigations are presented in this chapter:

1. The groups reporting severe hypoglycaemia (SH) at baseline or at year 4 and the respective remaining populations are compared in terms of demographics and clinical characteristics.

2. Results from the prospective 6-month survey of SH administered following the year 1 clinic are compared with the self-report of incident SH occurring between baseline and year 4 with the aim to evaluate the overall validity of the self-report measure in the ET2DS.

3. Hypoglycaemia associations with cognition function are investigated with SH treated both as a potential risk factor for poorer cognitive outcome, and as a potential consequence of poorer prior cognitive ability. In order to determine the independence of the findings from these analyses from potential influences by subjects with dementia diagnosis, the analyses of SH as a potential consequence of poorer cognitive ability and as a risk factor for poorer cognitive outcome (which was restricted to analyses of the four-year change in cognitive function – the main outcome of interest), were repeated with exclusion of dementia cases. Results from these analyses are shown in Appendix D; none of the findings reported in this chapter were altered in terms of significance levels or effect size in this step.

7.2 Risk factor profiles in subjects reporting hypoglycaemia

7.2.1 Risk factor associations with a lifetime history of severe hypoglycaemia

Individuals with a lifetime history of SH reported at baseline had longer disease duration, and were more likely to be female, on insulin treatment and to have lower blood pressure and poorer glycaemic control (Table 7.1). They also had increased prevalence of macro- and microvascular disease at baseline. Amongst the macrovascular disease variables, prevalence particularly of coronary heart disease appeared to be increased in the group with a baseline history of SH. Mean age, mean cholesterol, prevalence of symptomatic peripheral arterial disease and smoking history appeared to be equivalent in both groups. There was lower MMSE in participants with a baseline history of SH (geometric means 28.3 ± 1.1 versus 27.9 ± 1.1; p=0.023). History of SH was unrelated to the risk of dementia diagnosis by year 4 (n=19) (age- and sex-adjusted OR 1.76, 95% CI 0.23, 13.62; p=0.59).

Table 7.1: Baseline risk factors in individuals with a lifetime history of severe hypoglycaemia reported at baseline

	No severe hypoglycaemia (total n=929)	Lifetime history of severe hypoglycaemia (≥1 episodes) (n=112)	p-value for difference or trend
Age (years)	67.9 ± 4.2	67.9 ± 4.4	0.98
Male sex (%)	491 (52.9)	46 (41.1)	0.018
Duration of diabetes (years)	6 (3 – 10)	10 (6 – 15)	<0.001
Current treatment			<0.001
Insulin +/-tablets	130 (14.0)	51 (45.5)	
Tablets alone	611 (65.8)	53 (47.3)	
Diet alone	188 (20.2)	7 (6.3)	
HbA1c (%)	7.36 ± 1.06	7.66 ± 1.31	0.007
Systolic bp (mmHg)	133.9 ± 16.3	128.8 ± 17.23	0.002
Diastolic bp (mmHg)	69.5 ± 8.9	65.8 ± 9.4	<0.001
Macrovascular disease			
Myocardial infarction	120 (12.9)	27 (24.1)	0.001
Angina	238 (25.6)	50 (44.6)	<0.001
Stroke	50 (5.4)	11 (9.8)	0.06
TIA	23 (2.5)	6 (5.4)	0.08
Claudication	56 (6.0)	9 (8.0)	0.41
'Any MVD'	320 (34.4)	61 (54.5)	<0.001
Total cholesterol (mmol/L)	4.32 ± 0.90	4.16 ± 0.84	0.09
HDL cholesterol (mmol/L)	1.29 ± 0.36	1.29 ± 0.38	0.97
Smoking			0.41
Current	129 (13.9)	20 (17.9)	
Ex-smoker	441 (47.5)	47 (42.0)	
Never smoked	359 (38.6)	45 (40.2)	
DR severity			<0.001
No	632 (68.0)	57 (50.9)	
Mild	244 (26.3)	42 (37.5)	
Moderate/ severe	34 (3.7)	12 (10.7)	

Values are means ± SD, medians (interquartile range) or n (%). Log-transformed values were used for analyses of duration of diabetes. TIA, transient ischaemic attack; 'any MVD', any symptomatic macrovascular disease; DR, diabetic retinopathy; HDL, high-density lipoprotein; bp, blood pressure.

7.2.2 Risk factors associations with incident severe hypoglycaemia

The baseline characteristics associated with incident SH were found to be very similar to those identified to be associated with a baseline history of SH (Table 7.2), which may be attributed to large overlaps in participants between the two groups (27 of the 85 participants in the incident SH group had also had a lifetime history of SH at baseline). Subjects reporting incident SH tended to be female, had longer duration of diabetes, were likely to be on insulin treatment, had poorer glycaemic control, lower blood pressure and increased prevalence of TIA at baseline. Contrasting the findings for a baseline history of SH, incident SH was unrelated to the remaining macrovascular disease categories or to microvascular disease at baseline. Patients with incident SH also tended be younger and were likely to be smoking at baseline, despite age and smoking not being associated with a baseline history of SH (Table 7.1). Baseline MMSE scores were similar for the incident SH group and the remaining population (data not shown; p=0.64).

Table 7.2: Baseline risk factors in individuals with incident severe hypoglycaemia

	No severe hypoglycaemia (total n=730)	**Incident severe hypoglycaemia (≥1 epsiodes) (n=85)**	**p-value for difference or trend**
Age (years)	67.8 ± 4.2	66.7 ± 4.1	0.022
Male sex (%)	393 (53.8)	32 (37.6)	0.005
Duration of diabetes (years)	6 (3 – 10)	10 (5 – 15)	<0.001
Current treatment			<0.001
Insulin +/-tablets	103 (14.1)	31 (36.5)	
Tablets alone	471 (64.5)	47 (55.3)	
Diet alone	155 (21.2)	7 (8.2)	
HbA1c (%)	7.32 ± 1.06	7.86 ±1.22	<0.001
Systolic bp (mmHg)	132.8 ± 15.8	129.7 ± 15.4	0.09
Diastolic bp (mmHg)	69.2 ± 9.2	66.8 ± 6.2	0.002
Macrovascular disease			
Myocardial infarction	98 (13.4)	12 (14.1)	0.86
Angina	197 (27.0)	22 (25.9)	0.83
Stroke	42 (5.8)	2 (2.4)	0.19
TIA	19 (2.6)	7 (8.2)	0.005
Claudication	47 (6.4)	5 (5.9)	0.84
'Any MVD'	255 (34.9)	33 (38.8)	0.48
Total cholesterol (mmol/L)	4.32 ± 0.90	4.34 ± 0.80	0.89
HDL cholesterol (mmol/L)	1.29 ± 0.36	1.26 ± 0.35	0.35
Smoking			0.06
Current	88 (12.1)	18 (21.2)	
Ex-smoker	349 (47.8)	37 (43.5)	
Never smoked	293 (40.1)	30 (35.3)	
DR severity			0.23
No	493 (67.5)	50 (60.1)	
Mild	198 (27.1)	27 (31.8)	
Moderate/ severe	29 (4.0)	6 (7.1)	

Values are means ± SD, medians (interquartile range) or n (%). Duration of diabetes was log-transformed. TIA, transient ischaemic attack; 'any MVD', any symptomatic macrovascular disease; DR, diabetic retinopathy; HDL, high-density lipoprotein; bp, blood pressure.

7.3 Validation of self-reported hypoglycaemia in sub-study

As described in Chapter 5 (Results I), 45 episodes were reported by 30 subjects during the 6-month survey of SH carried out immediately after the year 1 clinic. Twenty-three of the 30 individuals with SH in the 6-month survey returned for the year 4 follow-up, and 17 (74%) there reported incident SH in the 4 years since baseline. Six individuals (26%) failed to recall that they had experienced SH since baseline, overall suggesting a reasonable level of accuracy in the recall of hypoglycaemic episodes.

7.4 Cognitive function as a risk factor for hypoglycaemia

To investigate the role of a lower cognitive function as a potential risk factor for hypoglycaemia, analyses of covariance (ANCOVAs) compared baseline MHVS (which is used here to estimate peak pre-morbid cognitive ability) and baseline global ability measured by *g* between the group with ≥1 episodes of incident SH and subjects who remained free of incident SH during follow-up. The odds ratios for incident SH of the respective lowest (versus the remaining two) tertiles of MHVS and baseline *g* were calculated.

7.4.1 Estimated peak pre-morbid ability and risk of hypoglycaemia

When treated as a continuous variable, baseline MHVS was not significantly related to a baseline history of SH (age- and sex-adjusted means in 'SH' group 30.26, 95% CI 29.28, 31.24 versus 31.06, 95% CI 30.72, 31.40 in 'no SH' group; p=0.13). However, individuals in the lowest tertile of the MVHS distribution (versus the remaining two tertiles) had significantly increased likelihood of having experienced SH prior to baseline (age- and sex-adjusted OR 1.55; 95% CI 1.04, 2.32, p=0.033).

Individuals who went on to experience incident SH between baseline and year 4, and the remaining sample also had similar baseline MHVS scores (age- and sex-adjusted mean 31.01, 95% CI 29.92, 32.10 versus 31.61, 95% CI 31.24, 31.98, p=0.30). The individuals in the lowest tertile of the MHVS distribution were not at increased risk of incident SH when compared with the remaining tertiles (age- and sex-adjusted OR 1.13, 95% CI 0.69, 1.83, p=0.63). These results were largely unchanged when analyses of baseline MHVS and incident hypoglycaemia were restricted to *first-ever* incident SH (adjusted mean MHVS in 'SH' group 30.58, 95% CI 29.20, 31.91 versus 31.69, 95% CI 31.31, 32.08 in 'no SH' group, p=0.11; age- and sex-adjusted OR 1.30, 95% CI 0.72, 2.33, p=0.39).

7.4.2 Baseline cognitive function and risk of hypoglycaemia

The group with incident SH had significantly lower global cognitive ability at baseline compared with the individuals free of incident SH (age- and sex-adjusted mean *g* -0.08, 95% CI -0.27, 0.12 versus 0.17, 95% CI 0.10, 0.24, p=0.019). The lowest tertile of baseline *g* was at increased risk of incident SH compared with the remaining tertiles (age- and sex-adjusted OR 2.04, 95% CI 1.25, 3.31, p=0.004). These findings remained statistically significant and even appeared to strengthen when analyses were restricted to risk of *first-ever* incident SH (age- and sex-adjusted mean *g* -0.08, 95% CI -0.33, 0.16 versus 0.18, 95% CI 0.12, 0.25, p=0.038; age- and sex-adjusted OR 2.46, 95% CI 1.37, 4.39, p=0.002), showing that the increased risk of hypoglycaemia in individuals with lower late-life ability was not driven by potential cognitive deficits caused by previous episodes.

7.5 Hypoglycaemia as a risk factor for poorer cognitive function

In the analyses on hypoglycaemia as a risk factor, a potential dose-response relationship between exposure to SH and cognitive outcome (level of cognitive function at year 4, estimated lifetime cognitive change, and four-year cognitive change) was initially explored by plotting each cognitive outcome against the four groups representing the frequency of hypoglycaemia (0, 1-2, 3-4 or ≥5 episodes prior to baseline or during follow-up). In subsequent ANCOVAs associating SH with each cognitive outcome, age and sex were controlled for in a first step, before conventional vascular and metabolic risk factors, macrovascular disease and microvascular disease were entered into the models with the aim to evaluate the independence of findings from these potential confounders. Unadjusted mean differences in cognitive test performance for the groups with and those without SH are shown in Appendix F.

7.5.1 Hypoglycaemia and cognitive function at year 4

Lifetime history of severe hypoglycaemia and level of cognitive function at year 4

Follow-up *g* was plotted against the four categories representing frequency of a lifetime history of hypoglycaemia reported at baseline (Figure 7.1a). Due to low participant numbers, confidence intervals were relatively large for the three groups reporting hypoglycaemia, and an age- and sex-adjusted ANCOVA showed that the number of episodes was overall unrelated to follow-up *g* ($F(3, 802) = 2.42$; $p=0.07$).

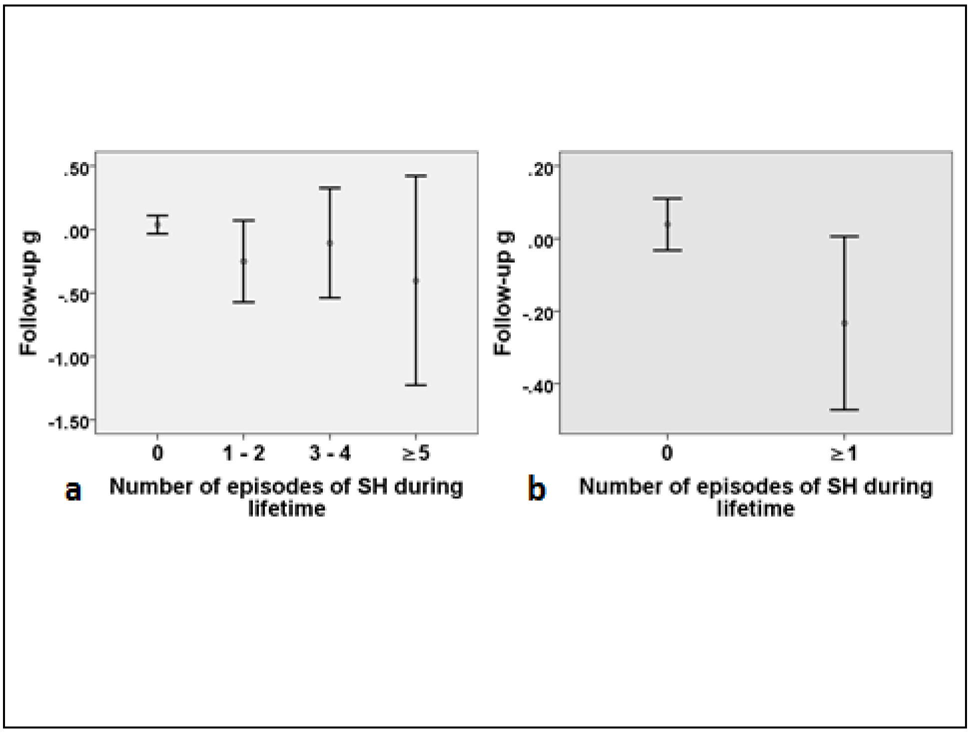


Figure 7.1: Follow-up *g* according to lifetime number of episodes of severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals)

With the aim of increasing statistical power, the groups with ≥1 episodes were collapsed (Figure 7.1b) and compared with hypoglycaemia-free individuals. The group without a history of SH outperformed the group with a baseline history of SH on *g* and on tests of reasoning (Matrix Reasoning), executive functioning (Trail-

Making Test-B) and processing speed (Digit Symbol Coding) (Table 7.3), including reaction time (Table 7.4). In age and sex-adjusted analyses, the significant group differences in cognitive test scores were of small to medium effect size (Cohen's $|d|$ = 0.24 to 0.39 for individual cognitive tests significantly associated with SH) (Cohen, 1992). Effect sizes were relatively largest for executive function, with SH explaining 1.4% of variance in Trail-Making Test-B performance. For the group with a history of SH, mean performance on the test was at around the 66th percentile of the group free of a history of SH. A history of SH accounted for 0.8% of variance in *g*; mean *g* for this group was at around the 62nd percentile of subjects free of a history of SH.

For *g* and Digit Symbol Coding, multivariable adjustment led to a p-value which was just short of statistical significance and for Matrix Reasoning the strength of the association was reduced but remained significant. For Trail-Making Test-B performance, the effect size was only marginally reduced and for reaction time tests even slightly increased in this modelling step. Thus, associations of hypoglycaemia and cognitive function with other clinical covariates, including micro- and macrovascular disease and conventional vascular and metabolic risk factors, did not appear to constitute major contributors to the observation of a lower cognitive function, particularly a lower processing speed measured by reaction time and lower executive function measured by the Trail-Making Test-B, in subjects with a history of SH compared with hypoglycaemia-free individuals.

Table 7.3: Lifetime history of severe hypoglycaemia and cognitive function at year 4

	No severe hypoglycaemia (n=739)	Severe hypoglycaemia (≥1 episodes; n=77)	p-value for difference	Partial η^2
Model 1: age, sex				
Matrix Reasoning	**11.77 (11.40 – 12.13)**	**10.14 (8.98 – 11.31)**	**0.009**	**0.008**
Letter-Number	9.01 (8.80 – 9.22)	8.42 (7.74 – 9.09)	0.103	0.003
Verbal Fluency	37.22 (36.29 – 38.14)	34.64 (31.72 – 37.56)	0.099	0.003
Digit Symbol Coding	**50.67 (49.67 – 51.66)**	**46.30 (43.19 – 49.41)**	**0.009**	**0.012**
Trail-Making	**109.07 (105.95 – 112.28)**	**128.64 (117.21 – 141.03)**	**0.001**	**0.014**
Faces	69.35 (68.76 – 69.94)	68.70 (66.85 – 70.56)	0.517	0.001
Logical Memory	27.28 (26.70 – 27.87)	27.71 (25.86 – 29.57)	0.664	<0.001
'g'	**0.04 (-0.03 – 0.11)**	**-0.26 (-0.49 – -0.04)**	**0.009**	**0.008**
Model 2[a]				
Matrix Reasoning	**11.81 (11.44 – 12.19)**	**10.47 (9.25 – 11.70)**	**0.042**	**0.005**
Letter-Number	9.03 (8.82 – 9.24)	8.50 (7.79 – 9.20)	0.158	0.003
Verbal Fluency	37.45 (36.52 – 38.38)	35.52 (32.48 – 38.56)	0.236	0.002
Digit Symbol Coding	50.65 (49.64 – 51.65)	47.71 (44.48 – 50.95)	0.091	0.004
Trail-Making	**108.42 (105.21 – 111.61)**	**125.09 (113.52 – 137.69)**	**0.006**	**0.010**
Faces	69.43 (68.83 – 70.04)	69.38 (67.41 – 71.35)	0.962	<0.001
Logical Memory	27.41 (26.81 – 28.01)	28.40 (26.47 – 30.33)	0.336	0.001
'g'	0.06 (-0.01 – 0.13)	-0.17 (-0.40 – 0.06)	0.061	0.005

Values are adjusted means (95% CI). N = 778 to 809 for Model 1; N = 743 to 769 for Model 2. Data for *g* have been imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Trail-Making, Trail-Making Test-B.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's d = 0.31 for *g*; d = 0.31 for Digit Symbol Coding; d = 0.32 for Matrix Reasoning; d = -0.39 for Trail-Making Test-B.

Model 2: Cohen's d = 0.19 for Matrix Reasoning; d = -0.34 for Trail-Making Test-B.

Table 7.4: Lifetime history of severe hypoglycaemia and reaction time at year 4

	No severe hypoglycaemia (n=739)	Severe hypoglycaemia (≥1 episodes; n=77)	p-value for difference	Partial η^2
Model 1: age, sex				
SRT mean	314.19 (309.51 – 318.62)	336.30 (320.54 – 352.48)	0.007	0.009
CRT mean	629.53 (620.43 – 638.64)	664.28 (634.13 – 694.44)	0.031	0.006
Model 2[a]				
SRT mean	313.88 (309.20 – 318.62)	337.65 (321.18 – 354.96)	0.006	0.010
CRT mean	628.72 (619.41 – 638.03)	666.11 (634.89 - 697.34)	0.025	0.007

Values are adjusted means (95% CI). N = 737 and 782 for Model 1; N = 702 and 745 for Model 2. SRT mean is log-transformed; means reported for this test are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = -0.24 for Choice Reaction Time; *d* = -0.31 for Simple Reaction Time.

Model 2: Cohen's *d* = -0.26 for Choice Reaction Time; *d* = -0.33 for Simple Reaction Time.

Incident severe hypoglycaemia and level of cognitive function at year 4

In an age- and sex-adjusted ANCOVA, the frequency of incident SH was found to be significantly associated with follow-up *g* ($F(3, 801) = 5.44$; $p=0.001$), but the association, which was again limited by large confidence intervals, did not appear to be linear (Figure 7.2a).

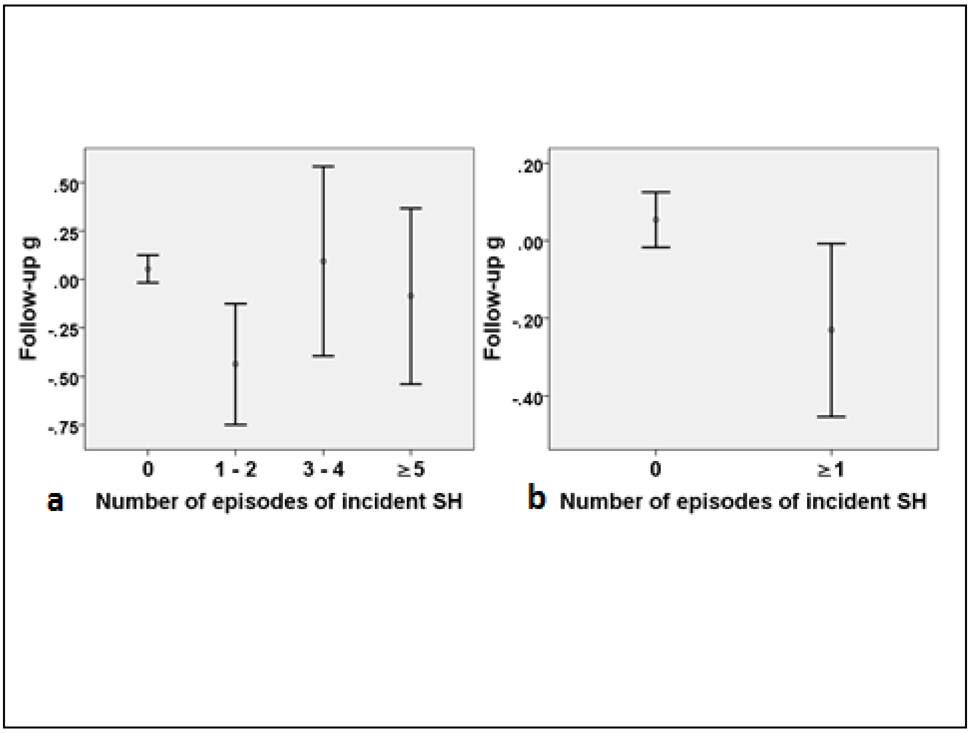


Figure 7.2: Follow-up *g* according to number of episodes of incident severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals)

With all participants with ≥1 episodes of incident SH grouped together in order to retain statistical power (Figure 7.2b), incident SH between baseline and year 4 was significantly and (compared with findings for a baseline history of SH) relatively strongly associated with lower performance on seven of the nine individual cognitive tests (all except Borkowski Verbal Fluency Test and Logical Memory), and with lower *g* (Table 7.5; Table 7.6). Effect sizes for tests significantly associated with incident SH were of small to medium effect sizes (Cohen's $|d|$ = 0.28 to 0.39) (Cohen, 1992), with relatively strongest findings for *g*. Hypoglycaemia accounted for 1.5% of variance in *g*; mean *g* of the group with incident SH was around the 66th

percentile of *g* in subjects free of incident SH. Effect sizes were somewhat attenuated when vascular risk factors, micro- and macrovascular disease were controlled for, but did not lose statistical significance, demonstrating that associations of incident hypoglycaemia with these potential confounders did appear to strongly contribute to the group differences in the level of late-life cognitive function. As shown in Appendix E, effect sizes and significance levels of the findings presented in this section were also largely unchanged when analyses were restricted to first-ever incident SH. The associations of incident SH and late-life cognitive function presented in this section therefore do not appear to be driven by those 27 subjects in the incident SH group who also had a baseline history of SH.

Table 7.5: Incident severe hypoglycaemia and cognitive function at year 4

	No severe hypoglycaemia (n=730)	**Severe hypoglycaemia (≥1 episodes; n=85)**	**p-value for difference**	**Partial ŋ²**
Model 1: age, sex				
Matrix Reasoning	**11.82 (11.45 – 12.19)**	**10.02 (8.92 – 11.13)**	**0.003**	**0.011**
Letter-Number	**9.07 (8.86 – 9.28)**	**8.19 (7.55 – 8.83)**	**0.011**	**0.008**
Verbal Fluency	37.22 (36.30 – 38.15)	35.36 (32.60 – 38.11)	0.208	0.002
Digit Symbol Coding	**50.84 (49.85 – 51.83)**	**46.74 (43.78 – 49.71)**	**0.010**	**0.008**
Trail-Making	**108.31 (105.21 – 111.50)**	**126.34 (115.82 – 137.83)**	**0.001**	**0.014**
Faces	**69.61 (69.02 – 70.20)**	**67.08 (65.33- 68.84)**	**0.008**	**0.009**
Logical Memory	27.43 (26.84 – 28.02)	26.77 (25.00 – 28.54)	0.493	0.001
'g'	**0.07 (0.00 – 0.13)**	**-0.33 (-0.53 – -0.12)**	**<0.001**	**0.015**
Model 2[a]:				
Matrix Reasoning	**11.83 (11.46 – 12.21)**	**10.55 (9.39 – 11.70)**	**0.039**	**0.006**
Letter-Number	**9.08 (8.86 – 9.29)**	**8.31 (7.65 – 8.97)**	**0.032**	**0.006**
Verbal Fluency	37.42 (36.49 – 38.35)	36.70 (33.85 – 39.54)	0.639	<0.001
Digit Symbol Coding	**50.84 (49.84 – 51.83)**	**47.76 (44.72 – 50.81)**	**0.062**	**0.005**
Trail-Making	**107.99 (104.90 – 111.16)**	**121.63 (111.16 - 133.09)**	**0.014**	**0.008**
Faces	**69.71 (69.10 – 70.31)**	**67.63 (65.78 – 69.47)**	**0.037**	**0.006**
Logical Memory	26.63 (27.03 – 28.23)	26.75 (24.92 – 28.59)	0.377	0.001
'g'	**0.08 (0.01 – 0.15)**	**-0.23 (-0.44 – -0.02)**	**0.008**	**0.009**

Values are adjusted means (95% CI). N = 776 to 808 for Model 1; N = 742 to 769 for Model 2. Data on *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Trail-Making, Trail-Making Test-B.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = 0.29 for Faces; *d* = 0.29 for Digit Symbol Coding; *d* = 0.32 for Letter-Number Sequencing; *d* = -0.36 for Trail-Making Test-B; *d* = 0.39 for *g*.

Model 2: Cohen's *d* = 0.22 for Digit Symbol Coding; *d* = 0.24 for Faces; *d* = 0.28 for Letter-Number Sequencing; *d* = -0.28 for Trail-Making Test-B; *d* = 0.31 for *g*; *d* = 0.36 for Matrix Reasoning.

Table 7.6: Incident severe hypoglycaemia and reaction time at year 4

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η^2
Model 1: age, sex				
SRT mean	**314.19 (309.51 – 318.94)**	**333.29 (318.62 – 348.63)**	**0.015**	**0.008**
CRT mean	630.41 (621.22 – 639.59)	658.57 (630.64 – 686.50)	0.061	0.005
Model 2[a]				
SRT mean	**315.58 (309.51 – 318.93)**	**330.63 (315.44 – 346.19)**	**0.043**	**0.006**
CRT mean	630.49 (621.10 – 639.88)	650.67 (621.74 – 679.61)	0.197	0.002

Values are adjusted means (95% CI). N = 736 and 781 for Model 1; N = 701 and 744 for Model 2. SRT mean is log-transformed; means reported for this test are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = 0.28 for Simple Reaction Time.

Model 2: Cohen's *d* = 0.24 for Simple Reaction Time.

7.5.2 Hypoglycaemia and estimated lifetime cognitive change

Lifetime history of severe hypoglycaemia and estimated lifetime cognitive change

The frequency of lifetime SH was overall significantly associated with the estimated change in cognitive function since peak pre-morbid ability as determined by adjustment of follow-up *g* for age, sex and baseline MHVS ($F(3, 792) = 3.42$; $p=0.017$), but again the finding suggested a non-linear relationship and was limited by large confidence intervals of the hypoglycaemia groups (Figure 7.3a).

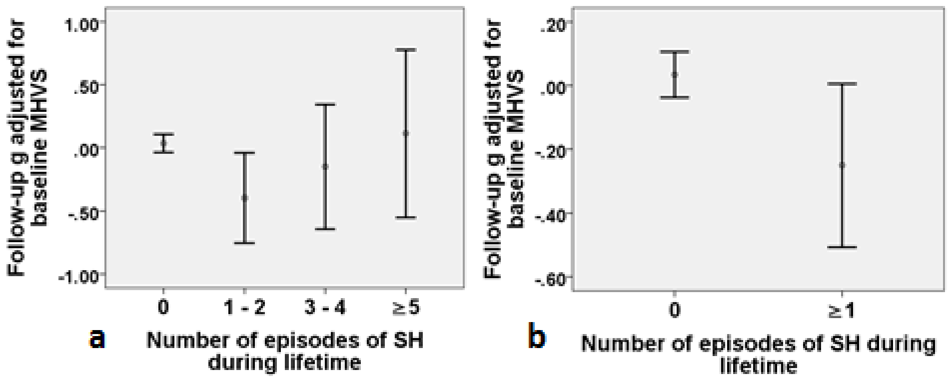


Figure 7.3: Follow-up *g* adjusted for baseline scores on the Mill-Hill Vocabulary Scale (MHVS) according to lifetime number of episodes of severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals) (error bars represent 95% confidence intervals)

Analyses comparing participants free of a lifetime history of SH with individuals with ≥1 episode (Figure 7.3b) showed significant associations between a lifetime history of SH and steeper estimated lifetime cognitive decline, which was statistically significant for the domains of reasoning (Matrix Reasoning), processing speed (Digit Symbol Coding; Choice Reaction Time; Simple Reaction Time), executive function (Trail-Making Test-B) and global ability measured by *g* (Table 7.7; Table 7.8). The associations were of small to medium effect size, with Cohen's |*d*| ranging between 0.26 and 0.37. Group differences were relatively largest for the Trail-Making Test-B of executive function. A history of SH accounted for 1.5% of estimated lifetime change in performance on this test and mean performance by the group with a history of SH was at around the 65th percentile of the Trail-Making distribution for the subjects free of a history of SH. These findings were largely independent of micro- and macrovascular disease or of vascular and metabolic risk factors). The group difference in *g*, for instance, remained relatively stable in terms of effect size (in age- and sex-adjusted model, Cohen's $d = 0.28$; in fully adjusted model $d = 0.27$); for the tests of reaction time effect sizes even slightly increased in this modelling step. Thus, individuals who were exposed to SH tended to experience a steeper cognitive decline between pre-hypoglycaemia peak pre-morbid ability and post-hypoglycaemia late-life cognitive function relatively independent of potential confounding by the remaining risk factors considered here.

Table 7.7: Lifetime history of severe hypoglycaemia and year 4 cognitive test scores adjusted for baseline MHVS

	No severe hypoglycaemia (n=739)	Severe hypoglycaemia (≥1 episodes; n=77)	p-value for difference	Partial η^2
Model 1: age, sex, baseline MHVS				
Matrix Reasoning	**11.72 (11.40 – 12.05)**	**10.41 (9.37 – 11.45)**	**0.018**	**0.007**
Letter-Number	9.01 (8.82 – 9.21)	8.47 (7.85 – 9.10)	0.105	0.003
Verbal Fluency	37.18 (36.33 – 38.03)	34.92 (32.23 – 37.60)	0.116	0.003
Digit Symbol Coding	**50.62 (49.70 – 51.54)**	**46.63 (43.74 – 49.52)**	**0.010**	**0.009**
Trail-Making	**109.07 (106.17 – 112.06)**	**127.61 (116.98 – 139.21)**	**0.001**	**0.015**
Faces	69.33 (68.76 – 68.89)	68.73 (66.94 – 70.52)	0.531	<0.001
Logical Memory	27.28 (26.76 – 27.81)	27.89 (26.23 – 29.55)	0.495	0.001
'g'	**0.04 (-0.02 – 0.10)**	**-0.24 (-0.42 – -0.06**	**0.004**	**0.010**
Model 2[a]:				
Matrix Reasoning	**11.80 (11.47 – 12.14)**	**10.41 (9.32 – 11.51)**	**0.018**	**0.007**
Letter-Number	9.05 (8.85 – 9.25)	8.42 (7.77 – 9.08)	0.074	0.004
Verbal Fluency	37.48 (36.61 – 38.35)	35.12 (32.30 – 37.94)	0.118	0.003
Digit Symbol Coding	**50.67 (49.73 – 51.61)**	**47.45 (44.43 – 50.47)**	**0.048**	**0.005**
Trail-Making	**108.31 (105.32 – 111.39)**	**126.09 (115.12 – 138.10)**	**0.002**	**0.013**
Faces	69.44 (68.86 – 70.03)	69.09 (67.18 – 71.00)	0.731	<0.001
Logical Memory	27.45 (26.91 – 27.99)	28.20 (26.46 – 29.94)	0.422	0.001
'g'	**0.06 (0.00 – 0.12)**	**-0.21 (-0.40 – 0.02)**	**0.008**	**0.009**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 770 to 800 for Model 1; N = 735 to 760 for Model 2. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Trail-Making, Trail-Making Test-B, MHVS, Mill-Hill Vocabulary Scale.

[a]model 1 + HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = 0.26 for Matrix Reasoning; *d* = 0.28 for *g*; *d* = 0.28 for Digit Symbol Coding; *d* = -0.37 for Trail-Making Test-B.

Model 2: Cohen's *d* = 0.23 for Digit Symbol Coding; *d* = 0.27 for *g*; *d* = 0.28 for Matrix Reasoning; *d* = -0.35 for Trail-Making Test-B.

Table 7.8: Lifetime history of severe hypoglycaemia and reaction time at year 4 adjusted for MHVS

	No severe hypoglycaemia (n=739)	Severe hypoglycaemia (≥1 episodes; n=77)	p-value for difference	Partial η²
Model 1: age, sex, baseline MHVS				
SRT mean	**314.51 (309.82 – 318.94)**	**336.30 (320.86 – 352.83)**	**0.007**	**0.009**
CRT mean	**629.55 (620.13 – 638.49)**	**665.61 (635.95 – 695.28)**	**0.023**	**0.007**
Model 2[a]				
SRT mean	**313.88 (309.20 – 318.62)**	**339.68 (323.11 – 356.74)**	**0.003**	**0.012**
CRT mean	**628.49 (619.33 – 637.65)**	**669.99 (639.21 – 700.76)**	**0.012**	**0.009**

Values are adjusted means (95% CI). N = 729 and 774 for Model 1; N = 694 and 737 for Model 2. SRT mean is log-transformed; means reported for this test are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time; MHVS, Mill-Hill Vocabulary Scale

[a]adjusted for age, sex, MHVS, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = -0.25 for Choice Reaction Time; *d* = -0.30 for Simple Reaction Time.

Model 2: Cohen's *d* = -0.29 for Choice Reaction Time; *d* = -0.36 for Simple Reaction Time.

Incident severe hypoglycaemia and estimated lifetime cognitive change

An ANCOVA with adjustment for age and sex revealed that the frequency of incident SH was significantly related to estimated lifetime cognitive change (F (3, 791) = 4.48; p=0.004), but again the pattern of results appears inconsistent, with large confidence intervals (Figure 7.4a).

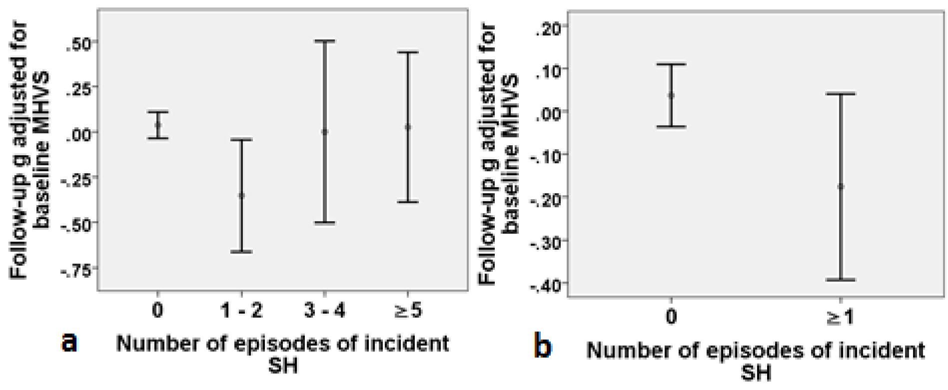


Figure 7.4: Follow-up *g* adjusted for baseline scores on the Mill-Hill Vocabulary Scale (MHVS) according to number of episodes of incident severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals)

Consistent with the observation in Figure 7.4b, analyses comparing subjects free of incident SH with those who experienced ≥1 episode between baseline and year 4 revealed that the incident SH group tended to have experienced steeper estimated lifetime decline in reasoning (Matrix Reasoning), processing speed (Digit Symbol Coding; Simple Reaction Time), executive function (Trail-Making Test-B), nonverbal memory (Faces) and global ability measured by *g* (Table 7.9; Table 7.10). Amongst all analyses presented in this chapter, this was also the only one to reveal statistically significant associations for the Letter-Number Sequencing Test of working memory. Effect sizes for statistically significant associations in age- and sex-adjusted analyses were of relatively small effect size (Cohen's |*d*| = 0.20 to 0.32). Again, associations were relatively independent from individual differences in micro- and macrovascular disease or vascular and metabolic risk factors, since associations of incident SH with estimated lifetime decline on *g* and on all cognitive tests with significant associations in the initial model, except Digit Symbol Coding, survived multivariable adjustment with relative preservation of effect sizes. The findings again were largely similar when analyses were restricted to first-ever incident SH (Appendix E), showing that subjects in this group who also had a baseline history of SH did not drive the findings presented in this section.

Table 7.9: Incident severe hypoglycaemia and year 4 cognitive test scores adjusted for baseline MHVS

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η²
Model 1: age, sex, baseline MHVS				
Matrix Reasoning	**11.78 (11.45 – 12.11)**	**10.23 (9.25 – 11.22)**	**0.004**	**0.011**
Letter-Number	**9.06 (8.67 – 9.25)**	**8.33 (7.75 – 8.92)**	**0.020**	**0.007**
Verbal Fluency	37.14 (36.29 – 37.99)	35.97 (33.43 – 38.51)	0.392	0.001
Digit Symbol Coding	**50.76 (49.84 – 51.69)**	**47.41 (44.65 – 50.16)**	**0.024**	**0.007**
Trail-Making	**108.53 (105.64 – 111.50)**	**124.21 (114.43 – 134.83)**	**0.002**	**0.012**
Faces	**69.58 (69.01 – 70.14)**	**67.24 (65.55 – 68.94)**	**0.011**	**0.008**
Logical Memory	27.38 (26.85 – 27.91)	27.36 (25.77 – 28.95)	0.986	<0.001
'g'	**0.06 (0.00 – 0.12)**	**-0.26 (-0.43 – -0.09)**	**0.001**	**0.015**
Model 2[a]:				
Matrix Reasoning	**11.81 (11.47 – 12.15)**	**10.66 (9.62 – 11.70)**	**0.041**	**0.006**
Letter-Number	**9.08 (8.88 – 9.28)**	**8.38 (7.77 – 9.00)**	**0.035**	**0.006**
Verbal Fluency	37.36 (36.50 – 38.23)	37.11 (34.46 – 39.76)	0.858	<0.001
Digit Symbol Coding	50.80 (49.86 – 51.74)	48.09 (45.24 – 50.95)	0.080	0.004
Trail-Making	**108.09 (105.11 – 111.16)**	**120.54 (110.61 – 131.24)**	**0.018**	**0.008**
Faces	**69.69 (69.10 – 70.27)**	**67.68 (65.89 – 69.48)**	**0.039**	**0.006**
Logical Memory	27.61 (27.07 – 28.15)	27.08 (25.42 – 28.74)	0.555	<0.001
'g'	**0.07 (0.02 – 0.13)**	**-0.19 (-0.37 – -0.14)**	**0.006**	**0.010**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 768 to 798 for Model 1; N = 734 to 760 for Model 2. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing; Verbal Fluency, Borkowski Verbal Fluency Test; Trail-Making, Trail-Making Test-B; MHVS, Mill-Hill Vocabulary Scale.

[a]model 1 +HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* = 0.20 for Faces; *d* = 0.24 for Digit Symbol Coding; *d* = 0.27 for Letter-Number Sequencing; *d* = 0.31 for Matrix Reasoning; *d* = 0.31 for *g*; *d* = -0.32 for Trail-Making Test-B. Model 2: Cohen's *d* = 0.23 for Faces; *d* = 0.23 for Matrix Reasoning; *d* = 0.25 for Letter-Number Sequencing; *d* = -0.26 for Trail-Making Test-B; *d* = 0.26 for *g*.

Table 7.10: Incident severe hypoglycaemia and reaction time at year 4 adjusted for MHVS

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η²
Model 1: age, sex, baseline MHVS				
SRT mean	**314.51 (310.13 – 319.29)**	**331.96 (317.67 – 346.89)**	**0.025**	**0.007**
CRT mean	630.72 (629.35 – 639.74)	656.62 (629.35 – 683.88)	0.078	0.004
Model 2[a]				
SRT mean	314.51 (309.82 – 319.26)	329.63 (314.82 – 345.16)	0.060	0.005
CRT mean	630.70 (621.45 – 639.96)	649.77 (621.40 – 678.15)	0.214	0.002

Values are adjusted means (95% CI). N = 728 and 773 for Model 1; N = 639 and 736 for Model 2. SRT is log-transformed; means reported for this test are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time; MHVS, Mill-Hill Vocabulary Scale.

[a]adjusted for age, sex, MHVS, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Model 1: Cohen's *d* for Simple Reaction Time = -0.26.

7.5.3 Hypoglycaemia and four-year cognitive change

Lifetime history of severe hypoglycaemia and four-year cognitive change

The frequency of a lifetime history of SH reported at baseline was found to be overall unrelated to the change in *g* between baseline and year 4 (age- and sex-adjusted $F(3, 801) = 1.91$; $p=0.12$; Figure 7.5a).

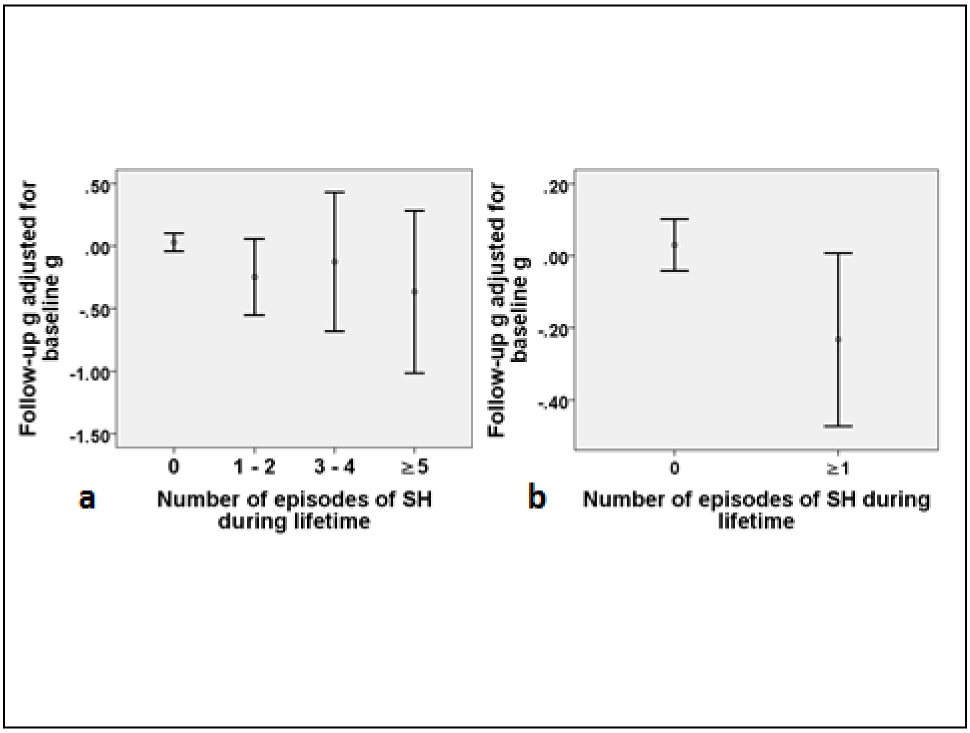


Figure 7.5: Four-year cognitive change according to lifetime number of episodes of severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals) (error bars represent 95% confidence intervals)

The results become clearer when statistical power is increased by collapsing the groups with ≥1 episodes (Figure 5b). Overall, this group tended to experience steeper decline in global ability measured by *g*, in reasoning (Matrix Reasoning) and in executive function measured by the Trail-Making Test-B over the course of the study (Table 7.11). Effect sizes were small (Cohen's $|d|$ = 0.20 for Trail-Making Test-B; 0.28 for *g*; 0.32 for Matrix Reasoning). Hypoglycaemia accounted for 0.7% of variance in four-year change in *g*, and mean *g* in the group with a history of SH was at around the 61^{st} percentile of the distribution of *g* of subjects free of a history of SH. The findings for *g* and for Matrix Reasoning were relatively independent from potential confounding by clinical covariates or by potential effects of peak pre-morbid ability, which was controlled for in a final step due to a potential for associations both with the risk of hypoglycaemia and with late-life cognitive decline. In fully adjusted models, Cohen's *d* was 0.26 for *g* and 0.30 for Matrix Reasoning, showing that subjects with a history of SH on average scored at around the 60^{th} percentile of the distribution of four-year decline in *g* and around the 62^{nd} percentile of the distribution of four-year decline in Matrix Reasoning scores of subjects who did not have a baseline history of SH.

Table 7.11: Lifetime history of severe hypoglycaemia and four-year cognitive change

	No severe hypoglycaemia (n=739)	Severe hypoglycaemia (≥1 episodes; n=77)	p-value for difference	Partial η^2
Model 1: age, sex, baseline score				
Matrix Reasoning	**11.78 (11.50 – 12.06)**	**10.19 (9.30 – 11.08)**	**0.001**	**0.014**
Letter-Number	8.99 (8.81 – 9.17)	8.72 (8.13 – 9.30)	0.369	0.001
Verbal Fluency	37.09 (36.55 – 37.63)	36.20 (34.50 – 37.89)	0.327	0.001
Digit Symbol Coding	50.44 (49.76 – 51.11)	48.54 (46.41 – 50.70)	0.096	0.004
Trail-Making	**109.73 (107.45 – 112.17)**	**119.34 (111.39 – 127.74)**	**0.024**	**0.007**
Faces	69.36 (68.88 – 69.84)	69.39 (66.88 – 69.91)	0.232	0.002
Logical Memory	27.40 (26.94 – 27.86)	27.30 (25.85 – 28.74)	0.896	<0.001
'g'	**0.03 (-0.04 – 0.10)**	**-0.25 (-0.48 – -0.02)**	**0.020**	**0.007**
Model 2[a]:				
Matrix Reasoning	**11.85 (11.57 – 12.14)**	**10.31 (9.37 – 11.26)**	**0.002**	**0.012**
Letter-Number	9.02 (8.84 – 9.21)	8.70 (8.09 – 9.31)	0.324	0.001
Verbal Fluency	37.38 (36.83 – 37.93)	36.49 (34.69 – 38.29)	0.356	0.001
Digit Symbol Coding	50.51 (49.81 – 51.20)	49.06 (46.82 – 51.30)	0.229	0.002
Trail-Making	109.07 (106.70 – 111.05)	116.51 (108.42 – 125.21)	0.089	0.004
Faces	69.48 (68.99 – 69.97)	68.73 (67.12 – 70.33)	0.383	0.001
Logical Memory	27.54 (27.07 – 28.00)	27.93 (26.42 – 29.44)	0.626	<0.001
'g'	**0.03 (-0.04 – 0.11)**	**-0.23 (-0.47 – 0.01)**	**0.040**	**0.006**
Model 3[b]:				
Matrix Reasoning	**11.83 (11.55 – 12.11)**	**10.32 (9.40 – 11.23)**	**0.002**	**0.013**
Letter-Number	9.04 (8.87 – 9.22)	8.61 (8.02 – 9.20)	0.171	0.003
Verbal Fluency	37.37 (36.82 – 37.92)	36.38 (34.59 – 38.18)	0.305	0.001
Digit Symbol Coding	50.51 (49.82 – 51.20)	48.97 (46.75 – 51.20)	0.198	0.002
Trail-Making	108.96 (106.59 – 111.39)	117.21 (109.07 – 125.96)	0.060	0.005
Faces	69.48 (68.99 – 69.96)	68.63 (67.03 – 70.23)	0.322	0.001
Logical Memory	27.55 (27.10 – 28.00)	27.93 (26.47 – 29.39)	0.627	<0.001
'g'	**0.04 (-0.04 – 0.11)**	**-0.23 (-0.47 – 0.00)**	**0.034**	**0.006**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 770 to 807 for Model 1; N = 736 to 768 for Model 2; N = 730 to 760 for Model 3. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing; Verbal Fluency, Borkowski Verbal Fluency Test; Trail-Making, Trail-Making Test-B; MHVS, Mill-Hill Vocabulary Scale.
[a]model 1 + HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.
[b]model 2 + baseline MHVS
Model 1: Cohen's *d* = -0.20 for Trail-Making Test-B; *d* = 0.28 for *g*; *d* = 0.32 for Matrix Reasoning.
Model 2: Cohen's *d* = 0.28 for *g*; *d* = 0.30 for Matrix Reasoning.
Model 3: Cohen's *d* = 0.26 for *g*; *d* = 0.30 for Matrix Reasoning.

Incident severe hypoglycaemia and four-year cognitive change

The frequency of incident SH during follow-up was found to be associated with concurrent cognitive change (age- and sex-adjusted $F(3, 800) = 4.60$; $p=0.003$). The finding was restricted by large confidence intervals (Figure 7.6a), and so groups with ≥1 episode of incident SH were collapsed (Figure 7.6b).

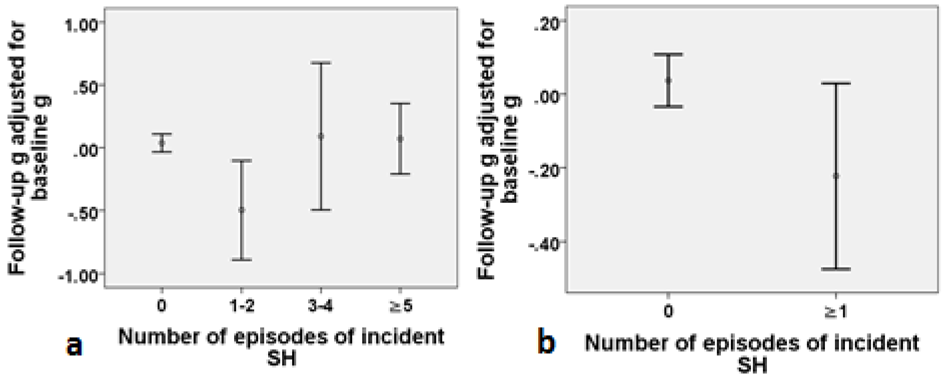


Figure 7.6: Four-year cognitive change according to number of episodes of incident severe hypoglycaemia (SH), categorised to 4 groups (a) and 2 groups (b) (error bars represent 95% confidence intervals)

The group with incident SH experienced steeper four-year cognitive decline compared with participants who remained free of SH (Table 7.12). Effect sizes were similarly small to those found for a lifetime history of SH (Table 7.11), with Cohen's $|d|$ ranging from 0.19 for decline in Digit Symbol Coding to 0.29 for decline in *g*. Compared with the findings for year 4 level of ability and estimated lifetime cognitive change, effect sizes were also overall weaker. Incident hypoglycaemia accounted for 0.9% of variance in four-year decline in *g*, and the group with incident SH on average scored at around the 61st percentile of the remaining sample's distribution of four-year decline in *g*. Findings somewhat attenuated but for Matrix Reasoning, Faces and for *g* remained statistically significant when potential clinical covariates were controlled for. Individual differences in estimated peak pre-morbid ability did not appear to play a role in the associations of incident hypoglycaemia and cognitive decline; for Matrix Reasoning and *g* the addition of MHVS to the model even increased effect sizes when compared with the previous modelling step (Cohen's $|d|$ = 0.24 and 0.25, respectively in fully adjusted models). Again, findings were largely unchanged when analyses presented in this section were restricted to first-ever incident SH (Appendix E).

Table 7.12: Incident severe hypoglycaemia and four-year cognitive change

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η^2
Model 1: age, sex, baseline score				
Matrix Reasoning	**11.78 (11.49 – 12.06)**	**10.58 (9.72 – 11.44)**	**0.010**	**0.008**
Letter-Number	9.04 (8.86 – 9.22)	8.56 (8.01 – 9.12)	0.102	0.004
Verbal Fluency	37.20 (36.66 – 37.74)	35.78 (34.17 – 37.93)	0.103	0.003
Digit Symbol Coding	**50.69 (50.01 – 51.37)**	**48.06 (46.03 – 50.10)**	**0.017**	**0.007**
Trail-Making	**108.96 (106.59 – 111.27)**	**119.10 (111.61 – 127.10)**	**0.011**	**0.008**
Faces	**69.55 (69.08 – 70.03)**	**37.38 (65.94 – 68.82)**	**0.005**	**0.010**
Logical Memory	27.45 (26.98 – 27.91)	27.25 (25.85 – 28.65)	0.791	<0.001
‘g’	**0.04 (-0.03 – 0.12)**	**-0.28 (-0.49 – -0.60)**	**0.006**	**0.009**
Model 2[a]:				
Matrix Reasoning	**11.82 (11.53 – 12.11)**	**10.85 (9.94 – 11.75)**	**0.046**	**0.005**
Letter-Number	9.05 (8.87 – 9.24)	8.63 (8.06 – 9.20)	0.173	0.003
Verbal Fluency	37.47 (36.20 – 38.03)	36.46 (34.76 – 38.16)	0.268	0.002
Digit Symbol Coding	50.74 (50.05 – 51.43)	48.61 (46.49 – 50.73)	0.063	0.005
Trail-Making	108.42 (106.06 – 110.83)	115.93 (108.31 – 124.09)	0.066	0.005
Faces	**69.66 (69.17 – 70.15)**	**67.87 (66.36 – 69.39)**	**0.029**	**0.006**
Logical Memory	27.62 (27.15 – 28.10)	27.47 (26.02 – 28.92)	0.843	<0.001
‘g’	**0.04 (-0.04 – 0.11)**	**-0.20 (-0.43 – 0.02)**	**0.049**	**0.005**
Model 3[b]:				
Matrix Reasoning	**11.80 (11.52 – 12.08)**	**10.86 (9.97 – 11.74)**	**0.048**	**0.005**
Letter-Number	9.07 (8.89 – 9.24)	8.64 (8.08 – 9.19)	0.150	0.003
Verbal Fluency	37.47 (36.91 – 38.02)	36.35 (34.66 – 38.05)	0.223	0.002
Digit Symbol Coding	50.74 (50.04 – 51.43)	48.65 (46.55 – 50.75)	0.066	0.005
Trail-Making	108.31 (105.95 – 110.72)	115.93 (108.31 – 123.97)	0.066	0.005
Faces	**69.64 (69.16 – 70.13)**	**67.94 (66.44 – 69.44)**	**0.035**	**0.006**

Logical Memory	27.64 (27.18 – 28.09)	27.48 (26.09 – 28.88)	0.838	<0.001
'g'	**0.04 (-0.03 – 0.12)**	**-0.22 (-0.45 – 0.00)**	**0.027**	**0.007**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 768 to 806 for Model 1; N = 735 to 768 for Model 2; N = 729 to 760 for Model 3. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making Test-B is log-transformed; means reported for this test are geometric means. Letter-Number, Letter Number Sequencing; Verbal Fluency, Borkowski Verbal Fluency Test; Trail-Making Test-B; MHVS, Mill-Hill Vocabulary Scale.

[a]model 1 + HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

[b]model 2 + baseline MHVS.

Model 1: Cohen's *d* = 0.19 for Digit Symbol Coding; *d* = -0.21 for Trail-Making Test-B; *d* = 0.24 for Matrix Reasoning; *d* = 0.25 for Faces; *d* = 0.29 for *g*.

Model 2: Cohen's *d* = 0.20 for Matrix Reasoning; *d* = 0.20 for Faces; *d* = 0.22 for *g*.

Model 3: Cohen's *d* = 0.19 for Faces; *d* = 0.24 for Matrix Reasoning; *d* = 0.25 for *g*.

7.5 Summary

Overall, the present analyses demonstrate a bidirectional relationship of hypoglycaemia with cognitive function. Subjects with a baseline history of severe hypoglycaemia had a 55% increased likelihood of having had a relatively low cognitive ability in young adulthood as estimated by the MHVS. A reduced late-life cognitive ability (although not a reduced estimated ability in young adulthood) also predisposed participants to an over two-fold increased risk of first-ever severe hypoglycaemia during the follow-up of the ET2DS. Additionally, a temporally relatively distant (lifetime history of SH) as well as a more recent (incident SH) experience of hypoglycaemia appeared to be linked to a lower level of late-life cognitive function in later life in associations of small to medium effect sizes. In addition to global ability measured by *g*, the cognitive domains most consistently associated with hypoglycaemia were non-verbal memory (Faces), executive function (Trail-Making Test-B), processing speed (Digit Symbol Coding; Choice Reaction Time; Simple Reaction Time) and non-verbal reasoning (Matrix Reasoning). On first inspection, somewhat larger effect sizes and a higher number of statistically significant cross-sectional associations with individual cognitive tests for analyses of a recent than of a more distant history of hypoglycaemia (which was particularly evident for the memory domain) could be seen to suggest that (should the present findings represent causality) hypoglycaemia affects cognitive function more severely in the short- than in the long-term. This would imply a potential for cognitive function to 'recover' following a hypoglycaemic episode.

However, the findings from subsequent analyses speak against this interpretation. The experience of hypoglycaemia either prior to or during the study period was linked to a steeper decline between estimated peak (pre-hypoglycaemia) cognitive function and (post-hypoglycaemia) assessment in later life, as well as to a steeper late-life cognitive decline, and the strength of associations were relatively independent of the amount of time that had passed between the hypoglycaemic event(s) and the cognitive assessment in later life.

Finally, the finding of relatively weaker associations for the rates of four-year decline than for estimated lifetime decline appear to mirror those reported for stroke in Chapter 6 (Results II) and show that the initial effects of hypoglycaemia on cognitive function appeared to be more severe than its effects on subsequent post-hypoglycaemia trajectories of cognitive decline.

Chapter 8: Results IV: Risk factors and cognition

8.1 Overview

Because participants of the ET2DS underwent extensive clinical characterisation, its data allows the concurrent analysis of a wider range of risk factors and their relationship with cognitive outcome. The aim of the first part of this chapter, which presents results from exploratory analyses, is to assess the relative importance of a range of risk factors in the determination of cognitive function and cognitive decline.

Initially, the risk factors to be used in the multivariate analyses of this chapter will be described, before bivariate associations amongst them are presented with the aim to set the background for the exploratory multivariable analyses associating risk factors with cognitive function and cognitive decline.

Due to some evidence which suggests that a number of risk factors may reflect the same underlying damage to the body (e.g., (T. Seeman et al., 2010)), the second part of the chapter will then evaluate the usefulness of a summary measure of 'allostatic load', which is derived from several risk factor measurements, and its relationship with later-life cognitive function and cognitive decline in the ET2DS.

8.2 Variables used in this chapter

A majority of the available baseline/ year 1 risk factor variables described in Chapter 5 (Results I) was used in the exploratory multivariable analyses presented in this chapter. Compared with Chapters 6 and 7 (Results II and III), some changes were applied to the risk factor variables in order to reduce the total number of risk factor variables (a, b, c), to maximise their validity (d, e, f) or to allow point-biserial or Pearson correlations (g, h). To summarise:

a) A binary coronary heart disease (CHD) variable was created to reflect the presence of MI and/or angina.
b) Intermittent claudication and/or ABI≤0.90 were summarised as a binary 'peripheral atherosclerosis (PA) variable.

c) Stroke was chosen as the only measure of cerebrovascular disease, as the validity of TIA may be questionable on the basis of possible misattribution of other age-related diseases to TIA.

d) Historical data on HbA1c and blood pressure obtained from the Lothian Diabetes Register replaced the variables measured at the clinic. Historical data may be more robust to confounding from factors associated with the clinic visit (e.g., heightened anxiety which may temporarily increase blood pressure), is less affected by intra-individual fluctuations and reflects the long-term exposure to the respective risk factor. Pearson correlations showed that the historical HbA1c variable correlated strongly and positively with HbA1c at the clinic visit ($r=0.70$; $p<0.001$) and moderately with plasma glucose at the clinic visit ($r=0.38$; $p<0.001$). Historical systolic blood pressure also correlated positively with systolic blood pressure measured at the clinic ($r=0.49$; $p<0.001$).

e) An LDL:HDL ratio was derived from the HDL and LDL variables. The ratio reflects the protective and detrimental contributions of each more appropriately and consequently has stronger predictive value for risk of cardiovascular disease when compared with non-ratio measures of cholesterol (e.g., HDL cholesterol) (Millán et al., 2006). As expected, LDL:HDL correlated positively with total cholesterol ($r=0.43$; $p<0.001$) and negatively with HDL cholesterol ($r=-0.66$; $p<0.001$).

f) A retrospective ‘packyears’ variable replaced the categorical smoking variable (never/ex/current). Packyears could be assumed to be a more accurate reflection of the lifetime exposure to the vascular burden and toxicity associated with smoking, because it takes into account a person’s complete smoking history and appears to have acceptable validity as determined by comparison with prospectively ascertained data on packyears (Bernaards, Twisk, Snel, Van Mechelen, & Kemper, 2001). Due to its continuous nature, the variable may also have increased statistical power over the categorical smoking variable. The distributions of packyears according to non-smokers, ex-smokers and current-smokers are shown in Table 8.1.

Table 8.1: Means packyears according to smoking group

	Mean ± SD
Never smoked (n=414)	0 ± 0
Ex-smoker (n=472)	25.70 ± 5.93
Current smoker (n=133)	35.91 ± 3.44

Packyears were square-root transformed.
Means and standard deviations were back-transformed.
SD, standard deviation.

g) In the bivariate associations presented in section 8.3, diabetic retinopathy (DR) was represented as a binary variable (any DR versus no DR). The original three-level categorical variable (moderate/severe DR versus mild DR versus no DR) was then used in the multivariable analyses of section 8.4.

h) In the correlations of risk factors presented in section 8.3, units of alcohol consumption in the previous year was used as a continuous variable, but in the multivariable analyses presented in section 8.4 was then analysed in terms of quintiles with the first quintile as abstainers. This is thought to be the most accurate reflection of the distribution of alcohol consumption in the sample and avoids the use of arbitrary thresholds, such as government guidelines (Conaglen, 2009).

Overall, the following 15 risk factors were included in the multivariable analyses presented in section 8.4: peripheral atherosclerosis (PA; presence of claudication and/or ABI≤0.90), coronary heart disease (CHD; angina and/or MI), stroke, cIMT, NT-proBNP, diabetic retinopathy (DR), packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, historical systolic blood pressure, historical HbA1c, inflammation factor, cortisol and baseline history of severe hypoglycaemia (SH). All except cIMT were measured at baseline; cIMT was measured at year 1. Incident SH was not considered in these analyses, because the focus was on the predictive ability of baseline/ year 1 risk factors, and because the correlation of baseline history of SH with incident SH would have led to difficulty in the modelling process (i.e., it is likely that even if both a history of SH and incident SH had made contributions to cognitive outcome, only one of the measures would be identified as a significant predictor in the model).

8.3 Inter-correlations amongst risk factors

The bivariate associations amongst the risk factors are shown in Table 8.2. To summarise:

- Individuals with higher inflammation tended to have poorer risk factor profiles throughout, with exception of non-significant associations with cIMT, retinopathy and waist-hip ratio.
- Many of the factors reflecting lifestyle (packyears, waist-hip ratio, LDL:HDL, alcohol) were interrelated; results were more inconsistent for blood pressure.
- People with higher depressive symptoms tended to have higher inflammation, atherosclerosis, higher likelihood of a history of severe hypoglycaemia, and poorer lifestyle, although depressive symptoms correlated negatively with alcohol consumption.
- Findings for CHD and stroke largely overlapped. In addition to inflammation, both were associated with higher waist-hip ratio, higher LDL:HDL and higher packyears, as well as with peripheral arterial disease, retinopathy and cardiac stress evident in raised NT-proBNP
- Higher alcohol consumption was negatively related to a majority of disease variables, but was linked to higher cIMT. Considering that this data is cross-sectional, the finding could either suggest protective effects of alcohol on disease processes or could show that people who are affected by disease stop drinking alcohol.
- Participants with a history of severe hypoglycaemia were likely to suffer from microvascular disease, tended to experience increased cardiac stress (NT-proBNP) and had lower blood pressure, higher inflammation and higher HbA1c.
- Cortisol stood out as relatively unrelated to all of the remaining risk factors, although individuals with higher cortisol tended to have raised inflammation and higher cholesterol levels.

Table 8.2: Univariate associations amongst risk factors

	HbA1c	WHR	LDL: HDL	Systolic bp	Pack-years	Alcohol units/yr	Corti-sol	PA*	cIMT	NT-proBNP	CHD*	Stroke*	DR*	SH*	TNF-α	Fibrin.	CRP	IL-6	Inflam-mation
HADS-D	**0.11 (<0.001)**	**0.10 (0.001)**	**0.10 (0.001)**	-0.03 (0.35)	**0.11 (0.001)**	**-0.11 (0.001)**	0.01 (0.71)	**0.12 (<0.001)**	**0.07 (0.051)**	**0.16 (<0.001)**	**0.19 (<0.001)**	**0.12 (<0.001)**	0.04 (0.24)	**0.15 (<0.001)**	**0.07 (0.034)**	**0.09 (0.004)**	**0.13 (<0.001)**	**0.14 (<0.001)**	**0.15 (<0.001)**
HbA1c	--	**0.11 (<0.001)**	**0.06 (0.053)**	**0.08 (0.011)**	0.00 (0.99)	**-0.14 (<0.001)**	0.00 (0.93)	**0.06 (0.039)**	0.02 (0.63)	0.00 (0.90)	-0.02 (0.58)	0.03 (0.34)	**0.25 (<0.001)**	**0.15 (<0.001)**	**0.10 (<0.001)**	**0.10 (0.001)**	**0.12 (<0.001)**	**0.12 (<0.001)**	**0.15 (<0.001)**
WHR		--	**0.16 (<0.001)**	0.04 (0.20)	**0.22 (<0.001)**	**0.15 (<0.001)**	-0.06 (0.06)	0.04 (0.23)	**0.15 (<0.001)**	-0.03 (0.34)	**0.16 (<0.001)**	**0.10 (0.001)**	**0.10 (0.001)**	0.02 (0.50)	0.05 (0.09)	-0.04 (0.18)	0.00 (0.90)	**0.09 (0.005)**	0.03 (0.37)
LDL: HDL			--	-0.01 (0.75)	0.05 (0.11)	-0.04 (0.18)	**0.13 (<0.001)**	0.03 (0.33)	**0.08 (0.014)**	0.01 (0.66)	**0.10 (0.002)**	**0.06 (0.045)**	0.01 (0.78)	-0.04 (0.16)	**0.14 (<0.001)**	0.00 (0.97)	**0.14 (<0.001)**	**0.08 (0.010)**	**0.12 (<0.001)**
Systolic bp				--	-0.06 (0.06)	0.00 (0.98)	0.04 (0.19)	0.06 (0.07)	**0.10 (0.003)**	0.02 (0.47)	**-0.08 (0.014)**	0.06 (0.07)	0.05 (0.08)	**-0.08 (0.008)**	0.05 (0.10)	0.02 (0.49)	0.042 (0.18)	**0.06 (0.043)**	**0.06 (0.048)**
Pack-years					--	**0.19 (<0.001)**	-0.01 (0.69)	**0.21 (<0.001)**	**0.13 (<0.001)**	0.02 (0.48)	**0.10 (0.001)**	**0.09 (0.006)**	-0.05 (0.13)	0.01 (0.83)	0.01 (0.69)	0.03 (0.32)	**0.07 (0.028)**	**0.12 (<0.001)**	**0.09 (0.005)**
Alcohol units/yr						--	0.05 (0.13)	**-0.07 (0.020)**	**0.07 (0.049)**	**-0.10 (0.001)**	-0.04 (0.19)	-0.02 (0.43)	-0.03 (0.28)	**-0.11 (0.001)**	**-0.10 (0.002)**	**-0.11 (0.001)**	**-0.10 (0.002)**	**-0.06 (0.045)**	**-0.12 (<0.001)**
Cortisol							--	0.03 (0.43)	-0.04 (0.20)	0.02 (0.56)	0.01 (0.86)	0.01 (0.73)	0.00 (0.90)	-0.04 (0.20)	0.00 (0.92)	0.05 (0.08)	**0.06 (0.053)**	**0.07 (0.027)**	**0.07 (0.027)**
PA*								--	**0.11 (0.001)**	**0.19 (<0.001)**	**1.83*** (<0.001)**	**2.61*** (<0.001)**	1.26*** (0.11)	1.31*** (0.20)	**0.07 (0.026)**	**0.16 (<0.001)**	**0.11 (<0.001)**	**0.12 (<0.001)**	**0.16 (<0.001)**
cIMT									--	**0.11 (0.001)**	**0.11 (0.001)**	**0.11 (0.001)**	0.05 (0.16)	0.01 (0.71)	0.03 (0.38)	-0.01 (0.75)	-0.02 (0.60)	0.05 (0.12)	0.02 (0.58)
NT-proBNP										--	**0.40 (<0.001)**	**0.14 (<0.001)**	**0.13 (<0.001)**	**0.10 (0.002)**	**0.17 (<0.001)**	**0.20 (<0.001)**	**0.11 (<0.001)**	**0.19 (<0.001)**	**0.24 (<0.001)**
CHD*											--	**2.71*** (<0.001)**	**2.32 (<0.001)**	1.16*** (0.31)	**0.11 (<0.001)**	**0.10 (0.001)**	**0.08 (0.010)**	**0.16 (<0.001)**	**0.16 (<0.001)**
Stroke*												--	**2.52 (0.001)**	1.92 (0.06)	0.01 (0.66)	**0.06 (0.046)**	0.05 (0.09)	**0.07 (0.029)**	**0.07 (0.017)**
DR*													--	**2.15*** (<0.001)**	**0.08 (0.012)**	**0.06 (0.051)**	**-0.06 (0.047)**	**0.06 (0.048)**	0.04 (0.25)
SH*														--	**0.11 (<0.001)**	**0.11 (0.001)**	0.04 (0.22)	**0.06 (0.036)**	**0.10 (0.001)**
TNF-α															--	**0.13 (<0.001)**	**0.12(<0.001)**	**0.31 (<0.001)**	**0.44****
Fibrin.																--	**0.54 (<0.001)**	**0.34 (<0.001)**	**0.76****
CRP																	--	**0.43 (<0.001)**	**0.81****
IL-6																		--	**0.76****

Analyses between continuous variables and between one continuous are two-tailed Pearson correlations. All risk factors except cIMT were measured at baseline; cIMT was measured at year 1. Analyses between one continuous and one categorical variable are point-biserial correlations. Analyses between two categorical variables are logistic regression analyses. Values are correlation coefficients (p-values).

All variables except cIMT measured at baseline; cIMT was measured at year 1. NT-proBNP, alcohol, TNF α, IL-6 and CRP are log-transformed. Packyears are square root-transformed. *binary categorical variables. **values are factor loadings. ***values are odds ratios. HADS-D, depression subscale of the Hospital Anxiety and Depression Questionnaire; CHD, coronary heart disease (myocardial infarction and/or angina); PA, peripheral atherosclerosis (intermittent claudication and/or ABI ≤0.90), bp, blood pressure; WHR, waist-hip-ratio; SH, baseline history of severe hypoglycaemia; fibrin., fibrinogen; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; CRP, c-reactive protein; LDL:HDL, low-density lipoprotein: high-density lipoprotein ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; DR, diabetic retinopathy; cIMT, carotid intima-media thickness; ‘inflammation’ is the inflammation ‘factor’ extracted from TNF- α, IL-6, CRP and fibrinogen. PA, DR, CHD and SH are binary variables.

8.4 Multivariable association of a range of risk factors with cognitive outcome

8.4.1 Stepwise linear regression method

The relationship of all of the risk factors with late-life cognitive outcome (follow-up *g*, estimated lifetime change *g*, four-year change in *g*) was investigated using a combination of hierarchical and stepwise regression procedures. In the first block of the regression model, the covariates to be controlled for were entered. The stepwise procedure was then applied to the second block of the analysis.

In a stepwise procedure, all ‘predictors’ under investigation are entered one at a time and the one predictor most strongly related to the specific outcome is revealed. All further predictors are then entered one at a time, and those with statistical significance above a pre-specified criterion are retained. As predictors became non-significant upon inclusion of another predictor, these are removed from the model. This automated process is terminated once no further significant predictors are found. The stepwise procedure is therefore a combination of the ‘forward selection’ (where predictors are added into the model one at a time with retention of significant predictors) and the ‘backward elimination’ approach (where the model starts out with all predictors and subsequently removes predictors one at a time where significance is below a given threshold) (Livingston, Cao, & Dimick, 2010). The stepwise procedure is advantageous in the analysis of a large number of predictors, because it reveals the contribution of each to the outcome in terms of the proportion of variance in the outcome measure accounted for. It further allows the identification of an ‘optimal’ model explaining the data.

The approach has also met with criticism in the literature, however. It has been noted that the selection of predictors in the stepwise procedure is flawed, because the procedure may underestimate standard errors and p-values and may result in ‘optimistic’, i.e. overestimated, beta coefficients (Pace, 2008; Wang, Zhang, & Bakhai, 2004). Consequently, stepwise regression has a high probability of ‘model overfitting’ due to the identification of spurious associations between predictors and outcome (Livingston et al., 2010). In one stepwise linear regression simulation, this occurred in one out of 20 predictors (Genell, Nemes, Steineck, & Dickman, 2010); another study on logistic stepwise regression found it to be the case in between 38% and 80% of simulations depending on sample size (Pace, 2008). This risk of error may be even higher when a redundant predictor is correlated with a true predictor (Genell et al., 2010). Consequently, there is an overall uncertainty on the contribution of the predictors, so that findings from a stepwise regression procedure on one population may not generalise to others, particularly when the data set is small. Despite these problems, researchers tend to implicitly interpret what they identify as the 'optimal' model as correct (D. Wang et al., 2004).

Yet, given that the stepwise regression approach is relatively common in the field of medical statistics (D. Wang et al., 2004), as well as in the literature investigating a number of potential predictors of cognitive outcome (Starr, Deary, Inch, Cross, & MacLennan, 1997; Stewart, Deary, Fowkes, & Price, 2006) or other health outcomes (Gale, Deary, Cooper, & Batty, 2012), and considering that the ET2DS has a relatively large sample size, stepwise regression is deemed suitable for the purpose of this chapter. The 'throw-in-everything' analyses presented here are intended as exploratory and all interpretations will be made with the consideration that the final model may not be an entirely accurate reflection of the underlying associations and generalisable to other populations. The findings should be seen as an initial contribution to the literature, which requires replication and validation in other, similarly large samples.

8.4.2 Multivariable association of risk factors with cognitive function at year 4

In a linear regression model of the relationship between all individual risk factors and follow-up *g*, age and sex were controlled for in a first block irrespective of whether or not their contributions to the model were statistically significant (Table 8.3). In the next, stepwise block, all of the aforementioned 15 risk factors (also listed in the footnote of Table 8.3) were entered in an automated fashion one at a time and retained in the model in cases of $p<0.05$. In cases where significance levels decreased following addition of other predictors in subsequent steps, previous predictors were removed from the model at $p>0.10$. Analyses were restricted to *g* rather than individual cognitive tests in order to limit the total number of analyses and because the relative predictive ability of risk factors with respect to the level of or changes in global ability are deemed most informative. Duration of diabetes and treatment mode were not controlled for with the aim to avoid over-adjustment. The final 'optimal' model of follow-up *g* is shown in Table 8.3.

Higher inflammation at baseline was the predictor which accounted for the largest proportion of variance in follow-up *g* with age and sex controlled for (3.7%). Higher packyears and higher waist-hip ratio also contributed significantly to the final model, although *lower* alcohol consumption was identified as a significant predictor of lower *g*. Contrasting the significant findings in the multivariate analyses presented in Chapters 6 and 7 (Results II and III), a baseline history of severe hypoglycaemia was not retained, and stroke was the only measure of macrovasuclar disease in the final model. It accounted for 0.7% of variance in *g* with all of the remaining predictors in the model held constant.

In order to establish the independence of these findings from potential confounding by depressed mood, the analysis was repeated with adjustment for baseline HADS-D in the block which also controlled for age and sex. The contributions of individual risk factors to *g* were close to identical to the age- and sex-adjusted model, although the total R^2 was slightly increased (Model 2; Table 8.3).

Table 8.3: Linear regression model of follow-up *g* on risk factors

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.27	0.01	<0.001	0.091
Sex*	0.08	0.08	0.089	
+inflammation factor	-0.14	0.04	<0.001	0.037
+alcohol	0.19	0.03	<0.001	0.027
+packyears	-0.13	0.01	<0.001	0.021
+waist-hip ratio	-0.14	0.55	<0.001	0.016
+stroke	-0.08	0.16	0.018	0.007
Total R^2				0.199
Model 2				
Age*	-0.27	0.01	<0.001	0.122
Sex*	0.09	0.09	0.038	
HADS-D*	-0.10	0.06	0.004	
+inflammation factor	-0.13	0.04	<0.001	0.028
+alcohol	0.19	0.03	<0.001	0.025
+packyears	-0.12	0.01	0.001	0.016
+waist-hip ratio	-0.13	0.56	0.002	0.012
+stroke	-0.07	0.16	0.036	0.005
Total R^2				0.209

N=672 for both models. *G* has been imputed. [a]statistics are shown for final respective model. [b]R^2 change following addition of predictor into the respective previous model. [*]entered in a first block. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears.

Variables entered in stepwise procedure: PA (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol, baseline history of SH.

CHD, coronary heart disease; PA, peripheral atherosclerosis; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia; HADS-D, Depression Subscale of the Hospital Anxiety and Depression Scale.

8.4.3 Multivariable association of risk factors with estimated lifetime change in cognitive function

Analyses of estimated lifetime change in *g* were performed using a stepwise linear regression procedure of follow-up *g*, which followed the adjustment for age, sex and baseline MHVS in a first block. This step determined that MHVS alone accounted for 27.6% of variance in follow-up *g*. The same 15 individual risk factors were again entered one at a time in an automated procedure and retained in the model if their association with was $p<0.05$. If subsequent addition of other predictors rendered associations of previously entered predictors non-significant at $p>0.10$, these were removed from the model. The final, optimal model identified in these analyses is shown in Table 8.4.

Although overall effect sizes were reduced, the significant contributors to the model of estimated lifetime change in *g* were largely similar to the model of follow-up *g*. The risk factors identified to contribute to the estimated lifetime change in *g* were inflammation, stroke, alcohol and packyears. Additionally, waist-hip ratio was no longer retained in the model and instead HbA1c was identified as a significant predictor. This could potentially be due to the correlation of HbA1c with waist-hip ratio ($r=0.11$; Table 8.2).

Again, inflammation had the relatively largest contribution. Higher late-life inflammation was associated with a steeper estimated lifetime cognitive decline, and accounted for 1.8% of the variance in this outcome with age and sex controlled for. A baseline history of stroke was also associated with significant decrements between estimated peak pre-morbid ability and post-stroke ability in later life, accounting for 0.6% of the variance. Again, higher packyears and lower alcohol consumptions were also significant predictors. Each accounted for 0.5% of additional variance following their respective addition to the model. HbA1c accounted for 1% of additional variance in estimated lifetime cognitive decline over and above age, sex and inflammation.

Again, neither a baseline history of severe hypoglycaemia nor macrovascular disease (except stroke) was retained in the model. Additional adjustment for HADS-D only marginally attenuated the contributions by significant predictors to the initial model (Model 2; Table 8.4).

Table 8.4: Linear regression model of estimated lifetime change in *g*

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.30	0.01	<0.001	0.117
Sex*	0.17	0.07	<0.001	
+inflammation factor	-0.11	0.03	0.001	0.018
+HbA1c	-0.09	0.03	0.004	0.010
+stroke	-0.07	0.14	0.018	0.006
+packyears	-0.09	0.01	0.006	0.005
+alcohol	0.08	0.02	0.016	0.005
Total R^2				0.161
Model 2				
Age*	-0.30	0.01	<0.001	0.128
Sex*	0.18	0.07	<0.001	
HADS-D*	-0.06	0.05	0.042	
+inflammation factor	-0.10	0.03	0.001	0.015
+HbA1c	-0.08	0.03	0.007	0.008
+stroke	-0.07	0.14	0.031	0.005
+packyears	-0.08	0.01	0.013	0.004
+alcohol	0.08	0.02	0.017	0.005
Total R^2				0.165

N=665 for both models. Baseline MHVS was entered in a first block (R^2 change = 0.276; this is not included in total R^2 shown in Table), so that the outcome is follow-up *g* adjusted for baseline MHVS. [a]statistics are shown for final model. [b]R^2 change following addition of predictor into the respective previous model.*entered in a second block. *G* has been imputed. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears. Variables entered in stepwise procedure: PA (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol; baseline history of SH.
CHD, coronary heart disease; PA, peripheral atherosclerosis; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia; HADS-D, Depression Subscale of the Hospital Anxiety and Depression Scale.

8.4.4 Multivariable association of risk factors with four-year cognitive change

The contributions of the range of risk factors to four-year change in cognitive function were also explored. With regression scores of follow-up *g* adjusted for baseline *g* as the outcome, age and sex were controlled for in a first block. In the stepwise part of the analysis, the 15 risk factors were entered one at a time in an automated procedure. These were retained if $p<0.05$ and removed if the level of significance dropped to $p>0.10$ following the addition of subsequent predictors. Results of the final model are shown in Table 8.5. As was also found for the level of cognitive function and for estimated lifetime cognitive change, inflammation and packyears were identified as significant predictors of the four-year change in *g*. HbA1c was also included, mirroring its significant contribution to the final model of estimated lifetime cognitive change. NT-proBNP and cIMT, neither of which had been included in the models of level of cognitive function and estimated lifetime cognitive change, were novel additions to the model.

Inflammation again was identified as the strongest predictor of four-year decline in *g*, and accounted for 2.2% of variance. Higher cIMT and higher NT-proBNP accounted for 1.6% and 0.6% if variance in four-year decline in *g* over and above the respective previously entered predictors. Higher HbA1c accounted for 1% of variance. Each standard deviation increase in HbA1c was associated with a 0.10 standard deviation decrease in follow-up *g* with baseline *g* and all of the remaining predictors held constant, demonstrating a relatively small effect size. In order to ascertain the independence of these findings from depressed mood, the analysis was repeated with HADS-D entered in a first block along with age and sex. Again, the findings were almost unchanged (Model 2; Table 8.5). Adjustment for MHVS along with age and sex in a first block aimed to explore the independence of the findings from individual differences in peak pre-morbid abilities, and slightly reduced the effect sizes of the final regression model of four-year change in *g* (Model 3; Table 8.5). In this step, the association of NT-proBNP became just short of statistical significance ($p=0.055$) and so this predictor was not retained in the final model. The variance explained by the remaining risk factors was largely unchanged, although it reduced notably, for inflammation, for instance, from 2.2% in age- and sex-adjusted analyses to 1.9% in analyses adjusted for age, sex and MHVS.

Table 8.5: Linear regression model of four-year change in *g*

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.10	0.01	0.015	0.022
Sex*	0.05	0.08	0.243	
+inflammation	-0.10	0.04	0.016	0.022
+cIMT	-0.12	0.22	0.003	0.016
+packyears	-0.11	0.01	0.007	0.011
+HbA1c	-0.10	0.04	0.007	0.010
+NT-proBNP	-0.08	0.03	0.037	0.006
Total R^2				0.086
Model 2				
Age*	-0.09	0.01	0.016	0.024
Sex*	0.05	0.08	0.261	
HADS-D*	0.02	0.06	0.690	
+inflammation	-0.10	0.04	0.015	0.020
+cIMT	-0.12	0.22	0.003	0.016
+packyears	-0.11	0.01	0.006	0.011
+HbA1c	-0.10	0.04	0.007	0.010
+NT-proBNP	-0.09	0.03	0.034	0.006
Total R^2				0.086
Model 3				
Age*	-0.12	0.01	0.001	0.051
Sex*	0.05	0.08	0.184	
MHVS*	0.15	0.01	<0.001	
+cIMT	-0.13	0.22	0.001	0.014
+inflammation	-0.10	0.04	0.011	0.019
+HbA1c	-0.10	0.04	0.008	0.010
+packyears	-0.09	0.01	0.019	0.008
+NT-proBNP	--	--	--	--
Total R^2				0.102

N=671 for Model 1 and Model 2. N=665 for Model 3. Outcome variable is follow-up *g* adjusted for baseline *g*. *G* has been imputed. [a]statistics are shown for final model. [b]R^2 change following addition of predictor into the respective previous model.
*entered in a first block. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears. Variables entered in stepwise procedure: PAD (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol, baseline history of SH.
CHD, coronary heart disease; PAD, peripheral arterial disease; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia.

8.4.5 Summary of the findings from multivariable analyses

In the stepwise regression analysis of 15 risk factor variables measured in the ET2DS, inflammation, smoking, alcohol intake, glycaemic control, abdominal obesity, stroke, cIMT and NT-proBNP were all identified as those with the strongest associations with cognitive outcome, as is illustrated in Figure 8.1. In all three final 'optimal' models of follow-up *g*, estimated lifetime decline in *g* and late-life decline in *g*, inflammation was the strongest contributor. Higher packyears, a higher waist-hip ratio and poorer glycaemic control were also linked to poorer cognitive outcome. Packyears was included in all three final models, HbA1c was included in the models of estimated lifetime and late-life cognitive change only, and the contribution of waist-hip ratio was limited to the cross-sectional analysis of the level of cognitive function at year 4. Higher alcohol consumption was associated with better level of late-life cognitive function and a shallower estimated lifetime cognitive decline, but was not associated with the four-year cognitive change in older age. Of note, age was identified as the single most important factor predicting late-life cognitive function, estimated lifetime cognitive change and late-life cognitive change. All results from the stepwise regression procedures were largely similar when participants with diagnosis of dementia were excluded from the analyses (Appendix D).

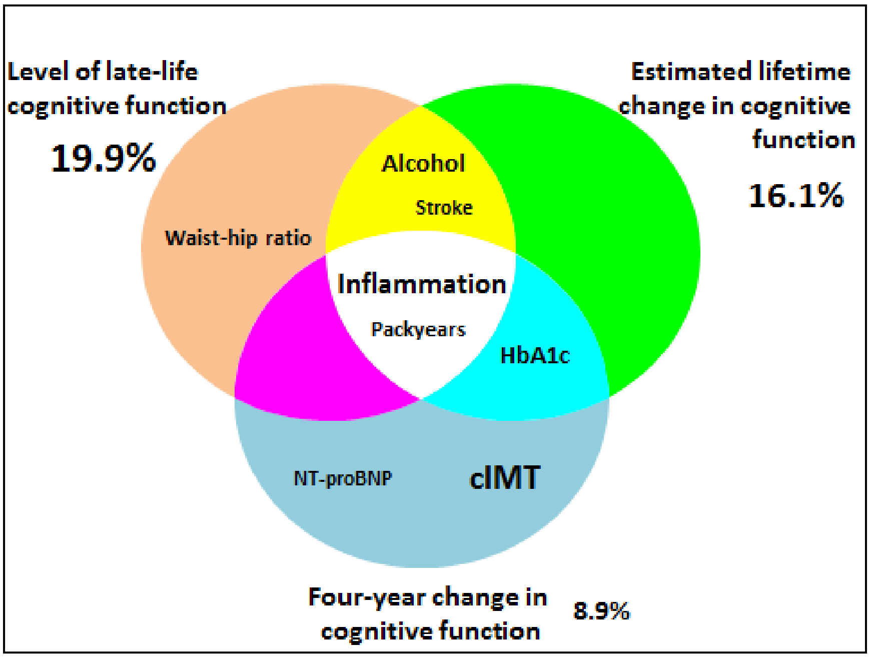


Figure 8.1: Venn diagram of significant predictors of cognitive outcome identified in the stepwise regression procedures. Note that these findings are strongly dependent on the variables entered into the modelling process as well as their inter-relationships, and so may not be definitive and replicable in another cohort. Font size is used to indicate relative effect sizes of associations. Percentages in brackets indicate total proportion of variance in respective model accounted for by the respective five risk factors, age and sex.

Neither the symptomatic macrovascular disease variables nor severe hypoglycaemia were significant contributors in the final model of four-year cognitive decline. For hypoglycaemia, this contrasts the findings described in Chapter 8 (Results III). For the macrovascular disease variables this is consistent with the findings in Chapter 7 (Results II), which implicated stroke as a predictor of steeper estimated lifetime cognitive change with a lesser role in the post-stroke cognitive change measured between baseline and year 4. In support of the findings of Chapter 7 (Results II), the subclinical markers NT-proBNP and cIMT were both identified as significant contributors in the final model of late-life cognitive decline. Although a lower ABI had been significantly associated with a steeper four-year cognitive decline in Chapter 7 (Results II), 'peripheral atherosclerosis', which captured subjects with subclinical (ABI ≤0.90) and/or symptomatic peripheral arterial disease (intermittent claudication), was not retained in the final regression model of four-year change in *g*, but this could potentially be due to reduced statistical power due to the categorical nature of the variable.

Chapter 9: Discussion

9.1 Overview

Using two waves of data from the Edinburgh Type 2 Diabetes Study (ET2DS), this thesis set out to investigate associations of macrovascular disease and severe hypoglycaemia with late-life cognitive decline short of frank dementia in older people with type 2 diabetes, with an attempt to explore potential underlying mechanisms through statistical adjustment for a wide list of potential confounders. Additionally, a statistical model was used to assess the relative contribution of a much wider range of potential risk factors to late-life cognitive decline, in a multivariable analysis fairly unique to the literature on human cognitive decline. In the present chapter, the main findings are discussed in the context of previous literature, before potential directions for future research, including investigations into possible targets for intervention, are described.

9.2 Main findings

In men and women aged 60-75 years with type 2 diabetes participating in the community-based Edinburgh Type 2 Diabetes Study (ET2DS), global cognitive function measured by *g* as well as ability in a number of age-sensitive cognitive domains declined over a four-year follow-up period. Individuals with a lower peak pre-morbid ability in young adulthood (estimated by a baseline test of vocabulary, MHVS) were more likely to have symptomatic macrovascular disease, evidence of subclinical macrovascular disease and to have experienced at least one episode of severe hypoglycaemia in later life (all assessed at baseline in the ET2DS). For severe hypoglycaemia, individuals with poorer global cognitive function at baseline were also at two-fold increased risk of having a first-ever severe hypoglycaemic episode over the subsequent four years.

The main outcome of interest for this thesis was the association of metabolic and vascular risk factors with subsequent, late-life cognitive decline. In this respect, stroke, subclinical macrovascular disease (measured by ankle-brachial index, ABI, circulating NT-proBNP or carotid intima-media thickness, cIMT), as well as a history of severe hypoglycaemia were all associated with a steeper decline in cognitive function since estimated peak pre-morbid ability and with a steeper four-year cognitive decline between baseline and four-year follow-up. For severe hypoglycaemia, incident hypoglycaemia episodes during the study period were also associated with steeper four-year cognitive decline. However, overall effect sizes of statistically significant findings were relatively small. This is likely to be due to a range of factors influencing and/or correlating with four-year cognitive decline. Consequently, individual measured risk factors then account for miniscule proportions of variance, as further discussed below in the section on findings from multivariable models. For instance, a history of hypoglycaemia reported at baseline and incident severe hypoglycaemia accounted for only 0.07% and 0.09% of variance in four-year decline in global ability measured by *g*, respectively. Similarly small effect sizes were also found for findings on macrovascular disease associations with cognitive decline. For stroke and for severe hypoglycaemia, associations were relatively more apparent for estimated lifetime cognitive decline, whereas for cIMT, NT-proBNP and ABI associations were more apparent for four-year cognitive decline, and only in these analyses were relatively independent of estimated peak pre-morbid cognitive function, socioeconomic status and stroke. In contrast, associations of these subclinical markers of macrovascular disease with level of late-life cognitive function and with estimated lifetime cognitive change appeared to stand under the confounding or mediation by these factors.

The exploratory analyses presented in the second part of the thesis assessed a total of 15 baseline metabolic and vascular risk factors in their relative contribution to a statistical model for late-life cognitive decline. The inflammation 'factor', extracted from circulating levels of four inflammatory markers, and packyears, reflecting the lifetime exposure to smoking, were identified as significant predictors in the 'optimal' models of global cognitive ability at year 4, of estimated lifetime change in cognitive function, and of four-year change in global ability. Inflammation was identified as the strongest predictor, and accounted for 3.7% of variance in follow-up cognitive function and for 2.2% in four-year cognitive decline with age and sex controlled for. Significant contributions of waist-hip ratio to the statistical models were restricted to the level of cognitive function at year 4, and contributions of stroke and alcohol consumption were limited to level of cognitive function at year 4 and estimated lifetime change in cognitive function. Long-term hyperglycaemia as measured by HbA1c contributed to the models of estimated lifetime cognitive change and of four-year cognitive change only. In the 'optimal' model of the latter, NT-proBNP and cIMT also contributed as significant predictors. For all risk factors (except alcohol which appeared to be protective), higher severity of disease or more detrimental lifestyle factors were associated with poorer cognitive outcome.

9.3 Study strengths

Due to its prospective study design, the ET2DS allowed the investigation of the relationship between baseline risk factors and subsequent cognitive change. The relatively large sample size of the study led to relatively high power of statistical analyses to detect actual associations present in the data, which depends strongly on the number of observations.

The sample recruited for the ET2DS was largely representative of all invited participants (Marioni et al., 2010). In contrast to other cohort studies often recruiting from a single city, recruitment was made from the population living in the city of Edinburgh and in rural areas in the Lothian area. The study population included participants from the full spectrum of community-living people with type 2 diabetes, with treatment modalities ranging from diet-controlled to insulin-treated. Overall, the study therefore should have acceptably high external validity, i.e. should be generalisable to the target population of all older people with type 2 diabetes living in Scotland (Szklo, 1998). Given the diabetic status of its participants, the retention rate of 78% between baseline and year 4, which is comparable to that of other prospective cohort studies (Kramer, von Muhlen, & Barrett-Connor, 2010; L. Wang et al., 2004) was very good. A large battery of well-validated cognitive tests free of floor and ceiling effects assessed performance on a range of cognitive domains. Influences by current mild hypoglycaemia on cognitive test performance were prevented by ensuring that blood glucose levels were well within the normoglycaemic range (≥4.0 mmol/l) prior to the start of cognitive test administration.

Participants were extensively characterised in terms of demographic and clinical risk factors, as well as cognitive testing, and hospital data were consulted to confirm diagnoses of clinical conditions including dementia and symptomatic macrovascular disease. All research staff were appropriately trained in their respective assessments, and cognitive test administration was validated prior to clinic begin by an external research associate. Standard operating procedures were followed to reduce observer bias. This detailed and accurate assessment of a wide range of potential confounding variables enabled high quality multivariable analyses to be undertaken.

In contrast to much of the previous literature, the ET2DS aimed to capture the entire range of age-related cognitive decline rather than focusing on the end-point of dementia, and, with only 19 participants identified as suffering from dementia by year 4, the study was relatively successful in this venture. Repetition of the main analyses with exclusion of dementia cases did not alter the statistical significance or effect size of findings, showing that dementia did not drive the risk factor associations with cognitive decline reported in this thesis.

Missing observations negatively impact on a study's statistical power to detect associations in the data. This may become problematic particularly in analyses involving a number of variables, such as the principal component analysis used to extract the general cognitive ability factor *g*, where analyses are then affected by an increased risk of Type II statistical error, i.e. the acceptance of a false null hypothesis. In the ET2DS, the loss of statistical power due to missing cognitive test data was strategically avoided through the use of standard operating procedures and an effort of testers to motivate participants. This resulted in relatively little data missing for cognitive tests at baseline (<2% for each test) and at year 4 (<5% for each test). Additionally, for the seven tests of fluid-type cognitive ability, missing data for participants with missing data on one, two or three tests was imputed prior to the calculation of *g*. The approach reduced the proportion of missing data on these tests to below 0.5% at baseline and to below 1% for the tests at year 4, and will have aided the successful detection of exposure-outcome associations in analyses of *g*.

9.4 Study limitations

9.4.1 Limitation of study design, risk factor variables and statistical analyses

Some limitations to the study design and the methodology of analyses presented in this thesis must be addressed. Prospective cohort studies are commonly vulnerable to a distortion of findings due to exclusion of individuals who are affected by the outcome of interest prior to study begin (left truncation), a failure to establish the outcome of interest by the end of the study (right censoring) and the inclusion of subjects who were affected by the outcome prior to study begin (left censoring). Left truncation leads to an underestimation of associations between predictors and outcome, for instance (Applebaum, Malloy, & Eisen, 2011; Cain et al., 2011). However, given the advanced age of subjects, who were recruited at an age at which cognitive declines tend to develop, as well as the continuous nature of the outcome of interest, these issues are unlikely to apply to the ET2DS. The study provided a 'snapshot' of age-related cognitive declines rather than focusing on incidence of a categorically assessed measure of cognitive function, such as the incidence of dementia.

Yet, the two-wave design of the study offering this 'snapshot' restricts the interpretation of cognitive 'change' of participants. Whereas a study with two waves may be successful in the estimation of the mean change of a sample between two time points, it may be less able to accurately capture person-specific changes in cognitive function, because the individual time paths for each participant are poorly defined (Rogosa, Brandt, & Zimowski, 1982; Wilson et al., 2002). This is due to inevitable influences of measurement error (further described in Section 9.4.2) on scores at each time point, which constitutes a kind of 'noise' masking the 'signal' under investigation, i.e. the longitudinal change in cognitive ability (Salthouse & Nesselroade, 2011). Studies with multi-wave designs (those with cognitive test data collected at three or more waves) are preferable to studies with two-wave designs, because measurement error is compensated for to an extent by repeated measurement of cognitive function. Yet, one group of researchers noted that "certainly, two waves of data are better than one” (Rogosa et al., 1982, p. 729), and the current findings may be seen as a first step towards the investigation of risk factor associations with cognitive decline in the ET2DS. Future waves of the study could now be used to confirm or refute the findings presented in this thesis.

Due to its observational study design, the present analyses were unable to evaluate the potential for causality in the reported associations, however, because none of the exposure variables were manipulated. As well as causal relationships, all associations of risk factors with cognitive decline reported in this thesis could therefore reflect associations of both with other, measured or unmeasured factors. The multivariable adjustment at least for a number of measured potential confounders aimed to explore this issue. The present findings, most of which survived multivariable adjustment, now merit further investigation, for instance in randomised controlled trials where possible, in order to identify potential causality underlying any associations.

Another limitation, which is similarly difficult to avoid, is the selective attrition between baseline and year 4, which may have systematically affected findings. Thus, year 4 data was ‘missing at random’ (but not ‘missing completely at random’), meaning that for each subject, data observed at baseline (including lower cognitive test scores), influenced the probability of missing data at follow-up (Dufouil, Brayne, & Clayton, 2004). This type of attrition will have selected the ‘attenders’ of the year 4 follow-up for high ability and will have led to an underestimation of cognitive declines during the study period. This was previously demonstrated in a cohort study reporting a substantial increase in ten-year cognitive declines following adjustment for drop-out (Chatfield, Matthews, Brayne, & Study, 2007).

The interpretation of findings is also limited by the specific risk factor variables used in this thesis. In contrast to cognitive test data, risk factor data were not imputed, and so any missing data on these parameters led to a disproportionate reduction in statistical power in analyses involving a number of risk factors. Specifically, this applied to the stepwise regression analyses associating 15 risk factors with cognitive outcome, which ‘lost’ around one fifth of the sample due to various missing risk factor data. Although the present analysis appears to have been unique in the use of an inflammation ‘factor’ (a single previous study had calculated a z-score from CRP and TNF-α; (Arfanakis et al., 2013)), findings are uninformative with respect to the contribution of individual markers. The use of the ‘packyears’ variable also did not allow an evaluation of associations of having ever smoked versus currently smoking with the risk of cognitive decline (Anstey, von Sanden, Salim, & O'Kearney, 2007). Alcohol intake was investigated in a linear fashion, despite the fact that the relationship of this risk factor with cognitive function appears to be complex and may be more appropriately represented by an inverted U-shaped function. Contrasting potential beneficial effects of moderate drinking compared with abstaining (Peters, Peters, Warner, Beckett, & Bulpitt, 2008), excessive drinking appears to have detrimental effects on cognition (Edelstein, Kritz-Silverstein, & Barrett-Connor, 1998; Hudetz & Warltier, 2007; Smith & Atkinson, 1995). However, strong contributions by excessive drinkers to findings, which could mask beneficial effects of moderate drinking in linear analyses, appear unlikely. All participants of the ET2DS were older and were suffering from diabetes, and only 5.6% reported ever having been told by a doctor that they had an alcohol problem.

Finally, with as many as 750 individual analyses reported in Chapter 6 (Results II) alone, the analyses presented in this thesis total thousands. One possibility to deal with the resulting high risk of Type I statistical error is to apply Bonferroni adjustment to p-values. As described in the Method chapter, this type of adjustment is often deemed too conservative (Perneger, 1998), including in this instance. The Bonferroni-adjusted p-value indicating statistical significance would be $p<0.00007$ for Chapter 6 alone, and (presumably) few or none of the analyses would survive this adjustment. However, the overall pattern of results suggests that a majority of findings with $p<0.05$ reflect more than chance results, so that the findings presented here likely to be relatively accurate reflections of risk factor associations with cognitive decline. None of the findings are interpreted as ‘correct’ and all merit further investigation, including attempts at replication.

9.4.2 Limitations of cognitive measurements

Cognitive test scores are generally influenced by error from a variety of sources, including test-specific measurement error. The MHVS, for instance, relies on literacy skills. The use of this test in particular was additionally restricted, because it represented a mere *estimate* of peak pre-morbid ability. With recruitment not made until later life, data on the actual level of cognitive function in early life was not available for the participants of the ET2DS. However, the MHVS has previously been shown to correlate strongly with more commonly used, well-recognised estimates of pre-morbid ability, such as the National Adult Reading Test (NART) (O'Carroll & Gilleard, 1986), and so its use was deemed acceptable for the purpose of the present analyses.

With exception of the reaction time test at year 4, each cognitive test was administered in a single trial at each wave. Because intra-individual variability appears to increase with age (Dykiert, Der, Starr, & Deary, 2012; West, Murphy, Armilio, Craik, & Stuss, 2002) and more severely in low-ability individuals (Rabbitt, Osman, Moore, & Stollery, 2001), it may have systematically affected test scores. Test scores may further be influenced by intra- and inter-tester variability in the ability to motivate participants and in the precision of test administration. For the MMSE, for instance, a previous study found that MMSE scores for identical subjects were dependent on testers (Fabrigoule, Lechevallier, Crasborn, Dartigues, & Orgogozo, 2003). No adjustments were made for blood glucose levels at the time of testing despite potential impacts of diurnal fluctuations in glycaemic control on cognitive test performance (McCall, 2005), potentially further contributing to measurement error affecting cognitive tests.

At their second visit to the research clinic, patients were also familiar with the clinic environment, the research staff and the experience, and in addition to an implicit learning of the testing material will have had the opportunity to develop strategies to improve performance. Such practice effects, which appear to eradicate only around seven years after initial exposure to the test material (Salthouse, Schroeder, & Ferrer, 2004), further (in addition to selective attrition) are likely to have added to an underestimation of the cognitive decline during the study period. For the tests of verbal and non-verbal memory (Faces, Logical Memory), practice effects may help to explain the significant improvements in scores between baseline and year 4.

Yet, with a previous report of significant improvements in cognitive test scores on as many as four of nine cognitive tests over similar length of follow-up (Fontbonne, Berr, Ducimetière, & Alpérovitch, 2001) (compared with two of seven cognitive tests in the ET2DS), it may be assumed that the underestimation of cognitive declines here were relatively limited. Additionally, the measurement error affecting the seven cognitive tests of fluid ability were partly compensated for by the calculation of a general ability factor *g*, and systematic influences by inter- and intra-tester variability in test administration and scoring appear unlikely given that standard operating procedures were followed and staff were appropriately trained in the administration of the cognitive test battery.

Overall, the ET2DS was affected by a number of limitations. Many, including potential influences by selective attrition, practice effects and an inability to reveal causality behind any associations, are inherent to all prospective cohort studies. The use of two-wave data further restricted the analysis of cognitive change due to measurement error affecting scores at each time point. However, all possible steps were taken to shield the analyses presented in this thesis from such influences.

9.5 Comparison of findings with previous studies, issue of confounding and potential mechanisms

In this section, the main findings presented in this thesis are described with reference to the previous literature. For the analyses of specific risk factors (macrovascular disease; severe hypoglycaemia), the roles of potential confounding factors and/or potential underlying mechanisms are evaluated.

9.5.1 Macrovacular disease and cognition

The findings from analyses of the relationship of macrovascular disease with cognitive function and cognitive decline during the course of the study are interpreted separately for risk factors representing symptomatic and those representing potentially subclinical levels of disease.

Symptomatic macrovascular disease and cognition

In contrast to the previous, limited literature on the relationship between coronary artery disease and cognitive ability in people with type 2 diabetes (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008; Cukierman-Yaffe et al., 2009; de Galan et al., 2009), angina and prior myocardial infarction (MI) were both associated with a lower level of late-life cognitive function. However, these finding appeared to be confounded by pre-morbid ability and lower pre-morbid ability itself appeared to predispose patients to late-life macrovascular disease as well as to lower ability in later life - it has been suggested that similar findings in previous studies may be due to poorer lifestyle, lower system integrity or reduced disease and injury prevention in lower-ability individuals (Batty, Deary, & Gottfredson, 2007). The present prospective analyses have extended current literature on type 2 diabetes, previously restricted to comparisons of the prevalence of CHD between cognitive ‘decliners’ and ‘non-decliners’ (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008; Reijmer et al., 2011), by showing that CHD was relatively unrelated to the subsequent rate of potentially more subtle cognitive decline. Together with the consistently non-significant findings for PAD, the results suggest that symptoms of macrovascular disease manifesting in areas other than the brain may not be useful risk markers for cognitive decline in people with type 2 diabetes.

Previous studies on cerebrovascular disease and cognition have mainly used data on stroke or infarction on brain imaging. In the ET2DS, stroke and transient ischaemic attack (TIA) were ascertained, but significant findings were restricted to stroke. This disparity is unexpected considering identical aetiology for stroke and TIA, but may be attributed to a relatively low number of participants in the TIA group (a relatively large proportion of attacks remain undiagnosed (Kessler & Thomas, 2009)) or to a common misdiagnosis (Prabhakaran, Silver, Warrior, McClenathan, & Lee, 2008). Stroke is already a well-established risk factor associated with cognitive dysfunction and impairment (Pendlebury & Rothwell, 2009), although the relationship with milder forms of impairment and cognitive decline in exclusively diabetic populations had previously not been extensively investigated. Consistent with some of the rare previous studies of people with type 2 diabetes applying detailed cognitive testing (Manschot et al., 2007; Manschot et al., 2006; Umemura et al., 2011), the level of processing speed, in addition to executive function and global ability measured by *g*, was most severely compromised in participants with a history of stroke. All associations survived adjustment for MHVS and were also independent of socioeconomic status measured by Scottish Index of Multiple Deprivation (SIMD) quintiles. This may demonstrate a fall in cognitive function at the time of the event, which did not completely recover to a pre-stroke level even over the course of at least four years. The overall weakness of effect sizes were perhaps surprising, however, when considering that the cerebrovascular accident(s) occurred in the brain, i.e. the 'substrate' of cognition, but may demonstrate a potential for the brain to recover parts of its function following stroke.

Conventional vascular risk factors, including blood pressure and smoking history, did not appear to drive the relationship between stroke and steeper estimated lifetime cognitive decline. Instead, the mechanisms by which the event affected cognitive function are likely to be direct. Stroke causes infarction in the area surrounding the occluded artery and the blood flow in this tissue is reduced to around 20% of previous flow (Sims & Muyderman, 2010). In addition to the resulting neuronal loss, infarcts increase oxidative stress (identified as a major cause of ageing (Kirkwood, 2005)) and initiate inflammatory responses (Barreto, White, Ouyang, Xu, & Giffard, 2011). In both humans and in animal models, stroke has also been linked to an increase in beta amyloid generation (Lee et al., 2005; Zhang et al., 2010), although, as aforementioned these potential immediate effects of stroke on cognition may have experienced improvement between the event and the cognitive assessment at the clinic, for instance through implicit compensation of deficits. Mediation of the decline between (estimated) pre- and post-stroke ability by vascular depression, which may be caused by vascular damage to frontal-subcortical circuits (Newberg, Davydow, & Lee, 2006) and manifests in symptoms similar to vascular dementia (Alexopoulos et al., 1997), appears unlikely given that the findings for stroke in the multivariable analysis of Chapter 8 (Results IV) survived post-hoc adjustment for depressive symptoms (HADS-D).

Contrary to two previous investigations in older adults with type 2 diabetes (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008; Reijmer et al., 2011), but consistent with one that had applied less detailed cognitive testing (Wu et al., 2003) and with evidence from the general population (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999; Kalmijn, Feskens, Launer, & Kromhout, 1996; Vermeer et al., 2003), a baseline history of stroke was associated with a steeper rate of late-life cognitive decline. Yet, these prospective associations were even weaker and less statistically significant compared with the analyses of estimated lifetime decline. Adjustment for MHVS (which predisposed participants to an increased risk of stroke as well as to a steeper late-life cognitive decline, consistent with at least *some* of the overall mixed evidence from the general population (Bourne, Fox, Deary, & Whalley, 2007; Gow et al., 2012)) led to statistical non-significance for *g*, and additional adjustment for SIMD further attenuated the finding. Overall, the evidence therefore suggests that the event weakly but negatively impacted on cognitive function, but played a lesser role in the determination of post-stroke cognitive trajectories.

Subclinical markers of macrovascular disease and cognition

The present investigation of NT-proBNP, cIMT and ABI was unique in two ways. It was the first prospective study of ABI and cIMT and cognitive decline measured prospectively by detailed neuropsychological tests in a population with type 2 diabetes, and was also the first to investigate NT-proBNP in any cognitive context in people with type 2 diabetes.

For NT-proBNP, cIMT and ABI, associations with lower level of ability were most consistently found for global ability measured by *g*, for various measures of the memory domain and for processing speed (associations with the latter as measured by reaction time were particularly strong for cIMT), but these findings appeared to be driven by socioeconomic status as well as by stroke. It may be the case that individuals with greater late-life atherosclerosis were likely to have come from a relatively lower socioeconomic background, to have had a history of stroke and (potentially due to these factors) were also likely to have a relatively lower late-life cognitive ability. Some previous studies have neglected the necessity of considering peak pre-morbid ability or its proxies in cross-sectional analyses of subclinical macrovascular disease (e.g., (Bruce, Davis, Casey, Starkstein, Clarnette, Foster, et al., 2008)). The present analyses have shown that the cross-sectional findings in the ET2DS were not driven by potential confounding by peak pre-morbid ability. Adjustment for estimated peak pre-morbid ability additionally allowed the analysis of risk factor associations with estimated lifetime change in cognitive function between young adulthood and later life. In associations of the subclinical markers of macrovascular disease (except those of cIMT) with this outcome, socioeconomic status and stroke appeared to play a confounding or mediating role. It is plausible, for instance, that a lower socioeconomic status may have predisposed people to an increased risk of late-life atherosclerosis of the heart and the periphery, an increased risk of stroke as well as to a steeper cognitive decline between peak pre-morbid ability and later life, although the data presented in this thesis does not allow conclusion as to specific underlying causal pathways. Atherosclerosis of the carotid artery (cIMT) appeared to play a role which was independent of socioeconomic status or of stroke, showing that this marker may be particularly valuable in the risk assessment of a patient's (estimated) lifetime cognitive change.

The previous prospective literature on NT-proBNP, cIMT or ABI and cognitive decline short of dementia had been severely restricted by flawed study designs, including extremely short follow-up periods (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008; McDonagh et al., 2010), the use of screening instruments (Kerola et al., 2010) potentially unsuitable for the detection of subtle cognitive declines (Tombaugh & McIntyre, 1992), the cognitive categorisation of participants and subsequent loss of statistical power (Bruce, Davis, Casey, Starkstein, Clarnette, Almeida, et al., 2008), or the focus on people with type 1 diabetes (Jacobson et al., 2011) despite their relatively young age, therefore neglecting the 'crucial period' of brain development in later life (Biessels, Deary, & Ryan, 2008). For ABI, inclusion of individuals with abnormally high measurements is also common in the literature despite the fact that due to high ABI reflecting stiffened arteries, their inclusion may weaken any linear analyses. This becomes apparent in a strengthening of findings on ABI-cognition links following the exclusion of high-ABI individuals (Feinkohl et al., 2013; Laurin, Masaki, White, & Launer, 2007).

With these potential flaws in previous studies addressed, the findings presented in this thesis have now demonstrated that higher circulating natriuretic peptides, higher cIMT and lower ABI at baseline are all relatively weakly but significantly associated with a steeper late-life cognitive decline in people with type 2 diabetes. Contrasting the evidence for symptomatic macrovascular disease, the associations of cognitive decline appeared slightly stronger for four-year cognitive decline compared with associations of these vascular markers with estimated lifetime cognitive decline. This pattern of results is of interest, because in terms of identifying elderly subjects at risk of subsequent cognitive decline, information on a patient's future decline may be more valuable compared with information which incorporates past decline.
The prospective observations were independent of potential confounding by estimated peak pre-morbid ability or of socioeconomic status.

Similar to *some* of the previous literature, which had considered the potential confounding role of stroke in associations of subclinical macrovascular disease with cognitive function or decline (Daniels et al., 2011; Kerola et al., 2010; Saleh, 2010), associations with estimated lifetime and late-life decline also either survived additional adjustment for stroke or fell just short of statistical significance. This demonstrates the usefulness of measuring late-life markers of atherosclerosis which may not have reached symptomatic levels. These markers appear to offer some, albeit relatively limited, information on the risk of future cognitive decline over and above the evaluation of a patient's potential history of stroke and stroke-associated neuronal damage, their pre-morbid ability or of their socioeconomic background. Their relationship with cognitive function was also not confounded by their associations with vascular risk factors, including smoking and hypertension, which themselves may either causally contribute to or correlate with poorer cognitive function.

Instead, the mechanisms relating NT-proBNP, ABI and cIMT to cognitive decline are likely to involve correlations of atherosclerosis in the periphery, the carotid artery and of the heart with atherosclerosis in the cerebral vasculature, including the risk of incident symptomatic or asymptomatic cerebral infarction (which was not considered in the present analyses). This explanation is supported by relatively largest effect sizes for cIMT, which is measured in relatively closest proximity to the brain, than for ABI or NT-proBNP. Additionally, all three markers were associated with slower processing speed (mirroring previous evidence, (Johnson, Price, Rafnsson, Deary, & Fowkes, 2010; Kindermann et al., 2012; Komulainen et al., 2007), which appears to be a typical early sign of dementia of the vascular type in particular (Kalaria & Erkinjuntti, 2006). Their roles as proxies of cerebral vascular pathology are also demonstrated in reports from brain imaging studies associating ABI (Bouchi et al., 2012), NT-proBNP (Nilsson, Gustafson, & Hultberg, 2012; Tabara et al., 2013) and cIMT (De Leeuw et al., 2000; Saleh, 2010; Takahashi et al., 2006) with symptomatic cerebrovascular disease, white matter heterogeneity, white matter lesions or leukoaraiosis.

Further potential mechanisms are marker-specific. Localised atherosclerosis of the carotid artery involves an increased risk of the formation of atherosclerotic plaques, which may become a source of thromboemboli resulting in cerebral infarction (Hollander et al., 2002). Raised circulating levels of natriuretic peptides are typical of congestive heart failure (CHF), which is associated with an increased risk of the development of cardiac mural emboli resulting in cerebral infarction (Bennett & Sauvé, 2003) and with cerebral hypoperfusion due to poor cardiac output (Bennett, Sauvé, & Shaw, 2005). Some evidence also points to direct effects of the peptide on endothelial dysfunction (Chong et al., 2004; van der Zander, Houben, Kroon, & de Leeuw, 1999). Although natriuretic peptides appear to be unable to pass the blood-brain barrier (Ermisch, Rühle, Kretzschmar, & Baethmann, 1991), it may be plausible that the blood-brain barrier is vulnerable to their passage when BBB function is compromised, as is the case in diabetes (Hawkins, Lundeen, Norwood, Brooks, & Egleton, 2007). This suggestion is highly speculative and the present observational study of course does not allow evaluations of any of the aforementioned potential pathophysiological mechanisms.

Integration of findings for symptomatic and subclinical macrovascular disease, and implications

Overall, the present results provide additional evidence that the impact of vascular disease on cognition may not be restricted to localised cerebral small vessel disease or altered blood flow and ischaemic damage as a consequence of stroke, and that cognitive decline could reflect systemic atherosclerotic changes. NT-proBNP, ABI and cIMT appear to function as biomarkers of risk of late-life cognitive decline, despite their strong associations with subclinical macrovascular disease in different areas of the vasculature and may offer *some* valuable information over and above traditional vascular risk factors. Stroke-associated declines in cognitive function were relatively limited considering the role of the brain as the 'substrate' for cognition, and were largely restricted to declines between (estimated) pre-stroke ability and post-stroke ability in later life. Associations of stroke with four-year cognitive decline was relatively weaker; none of the remaining measures of symptomatic macrovascular disease (MI, angina, PAD) were related to this outcome. Consequently, determination of subclinical macrovascular disease using continuous measures may be preferable over symptomatic categorically assessed macrovascular disease for the early identification of individuals at risk of late life cognitive decline. With the proximity of the carotid artery to the brain, cIMT could be hypothesised to represent the most accurate, non-invasive marker of asymptomatic cerebral vascular damage. In people with stroke, the monitoring of cognitive development may only be necessary in the short-term and in consideration of pre-stroke ability (estimated for instance by MHVS, occupation or relative report) to ascertain the damage caused by the event. However, it must be recognised that the categorical nature of 'events' data compared with the continuous distribution of the subclinical measures is likely to have influenced the relative levels of statistical significance for these variables.

9.5.2 Severe hypoglycaemia and cognition

Considering that all analyses of severe hypoglycaemia (SH) in the ET2DS were based on self-report, the validation of this data was paramount to ensure the validity of the study's findings. The 6-month survey of SH embedded in the main study aimed to address this issue. Its findings are initially described, before associations of SH with cognition are discussed in the context of previous literature. An attempt is made to account for any disparities of the present findings with those described in previous studies.

Validation of self-report data in the ET2DS

The blood glucose measurements reported during the 6-month survey of SH indicated that participants successfully identified severe episodes all of which appeared to have remained short of coma. The comparison of the retrospective recall of SH at year 4 with the prospectively ascertained data in the 6-month survey of SH further showed that 74% of the participants accurately recalled the experience of severe hypoglycaemia around four years after the episode had occurred. Two previous studies which, like the ET2DS, ascertained hypoglycaemia prospectively by telephone report and response to bi-monthly questionnaires, found that as many as 94% of patients with type 2 diabetes (Akram, Pedersen-Bjergaard, Carstensen, Borch-Johnson, & Thorsteinsson, 2009) and 90% of patients with type 1 diabetes (Pedersen-Bjergaard, Pramming, & Thorsteinsson, 2003) accurately recalled their experience of SH. However, recall in these studies will have been facilitated by short follow-up of one year which contrasts around three years in the present study.

Bidirectional relationship of severe hypoglycaemia with cognitive function

Even moderate hypoglycaemia is known to generate cognitive symptoms (Suh, Hamby, & Swanson, 2007), but potential long-term effects of severe episodes on brain function were at the centre of this thesis. A number of cross-sectional studies (Bruce et al., 2009; Punthakee et al., 2012) and cross-sectional analysis of the ET2DS itself (Aung et al., 2012) have previously demonstrated a relationship between hypoglycemia and poorer cognitive function in people with type 2 diabetes. However, there has been uncertainty about the reasons for this association and the direction of any possible causal relationship between hypoglycaemia and cognitive decrements. The following sections discuss the evidence from the present analyses and from the previous literature separately for hypoglycaemia as a potential risk factor for cognitive decline and as an event which occurs subsequent to poorer initial cognitive function.

Reduced cognitive function predicted incidence of severe hypoglycaemia

This appears to be the first analysis of the relationship between hypoglycaemia and peak pre-morbid ability in young adulthood as estimated by a vocabulary-based test in older patients with type 2 diabetes. Similar to a study of people with type 1 diabetes which estimated peak pre-morbid ability by the National Adult Reading Test (NART) (Deary et al., 1993; Deary, Langan, Graham, & Hepburn, 1992), baseline MHVS was unrelated to the risk of incident SH during the four years of follow-up in the ET2DS. This could be due to limited statistical power to detect associations based on the relatively short period of follow-up, which resulted in incident SH occurring in only 85 subjects. Compared with analyses of incident SH, statistical power was increased in the analyses of a lifetime history of SH reported prior to baseline, which affected 112 participants. Here, a lower baseline MHVS (relative to the distribution) was statistically significantly related to a 55% increased likelihood of having experienced SH prior to baseline, overall suggesting that a lower peak pre-morbid ability in young adulthood may predispose individuals to an increased risk of hypoglycaemia.

Patients with reduced late-life global function (relative to the distribution) were also at two-fold increased risk of incident SH during follow-up. This part of the results supported a number of previous studies reporting a two- to three-fold increased risk of hypoglycaemia during the course of three to twelve years in lower-functioning individuals with type 2 diabetes (Bruce et al., 2009; de Galan et al., 2009; Punthakee et al., 2012; Yaffe et al., 2013). In the light of the apparent bidirectional relationship of cognitive function with hypoglycaemia, which is described below, it is crucial to consider the potential for such associations to be driven by recurrent episodes. Prior to the present analysis (which found that the two-fold increased risk persisted and even slightly increased in effect size), ACCORD-MIND had been the only previous instance which investigated associations of cognitive function with the risk of *first-*

ever incidence of hypoglycaemia (Punthakee et al., 2012). Basis for the increased risk of hypoglycaemia in lower-functioning individuals may be a lower ability to adhere to prescribed treatment, to recognise hypoglycaemia, to respond appropriately when it occurs or to prevent hypoglycemia through modification of diabetes therapy. The evidence from children with type 1 diabetes (McNally, Rohan, Shroff Pendley, Delameter, & Drotar, 2010) and from other, older, patient populations (Alosco et al., 2012) supports links of lower cognitive function with poorer treatment adherence, although this does not appear to have been studied in people with type 2 diabetes.

Severe hypoglycaemia as a risk factor for cognitive decline

Compared with the evidence of lower cognitive function as a risk factor for subsequent hypoglycaemia and of lasting cognitive deficits following hypoglycaemic episodes resulting in coma (Chalmers et al., 1991; Iino et al., 2000; Xu et al., 2011), to date there has been less epidemiological evidence to support the hypothesis that severe hypoglycemia, which usually remains short of coma, may have a direct or indirect effect on the brain resulting in cognitive decrements.

In adults with type 1 diabetes, some evidence suggests that people with a history of SH may experience an accelerated estimated lifetime decline in cognitive function (Deary et al., 1992; Langan, Deary, Hepburn, & Frier, 1991), but the balance of cross-sectional (Brands, Biessels, De Haan, Kappelle, & Kessels, 2005) and prospective evidence, including from the DCCT/EDIC, has shown that SH may not affect cognitive function in this way (Reichard, Pihl, Rosenqvist, & Sule, 1996; The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group, 2007). However, comparison of findings between people with type 2 diabetes, including those participating in the ET2DS, and patients with type 1 diabetes may not be appropriate. Patients with type 1 diabetes are typically younger and have a lower prevalence of co-morbidities (Plotnikoff et al., 2007). Additionally, the DCCT cohort was atypical in that the participants had been selected for having high compliance with treatment and a low risk of hypoglycemia, such that these findings were restricted by low prevalence of both the risk factor (hypoglycemia) and the outcome (age-related cognitive decline).

In type 2 diabetes, the experience of hypoglycemia appears to be a risk factor for future dementia (Lin & Sheu, 2013; Whitmer, Karter, Yaffe, Quesenberry, & Selby, 2009; Yaffe et al., 2013). With dementia at the end-point of the spectrum of cognitive decline, associations of hypoglycaemia with subsequent cognitive impairment short of dementia are to be expected. The present analysis was the first to associate hypoglycaemia with potentially subtle cognitive decline in an observational analysis. It has provided the most robust evidence to date that exposure to at least one episode of severe hypoglycemia either preceding or concurrent with, change in cognition during ageing, is associated with an increase in the rate of estimated lifetime cognitive decline since peak pre-morbid ability as well as in the late-life cognitive decline short of frank dementia. Effects sizes were overall modest, with SH accounting for <1% of variance in four-year decline in *g*. The mean decline in *g* of the groups with hypoglycaemia was at around the 60th percentiles of the mean decline of the respective group free of hypoglycaemia. Relatively stronger associations with estimated lifetime than with four-year cognitive decline suggest that short-term effects of pre-episode to post-episode declines were more severe compared with long-term post-episode cognitive declines. Reasoning, executive function, processing speed and non-verbal memory appeared particularly vulnerable. The finding for the memory domain is consistent with published evidence for associations of hypoglycaemia with dementia (e.g., (Yaffe et al., 2013)), which is commonly preceded by memory impairment (Petersen, 2004). Unfortunately, it was not possible to investigate potential dose-response relationships due to few participants having been exposed to three or more episodes in the ET2DS.

A single previous investigation with observational study design had been performed on the topic, and had revealed no statistically significant links between hypoglycaemia and the risk of conversion between normal functioning, impairment short of dementia and frank dementia during two-year follow-up (there was even a non-significant trend for *higher* prevalence of hypoglycaemia in participants who converted compared with those who did not) (Bruce et al., 2009). The focus of the ET2DS on cognitive decline in the dementia-free range as well as its greater power to detect associations due to the treatment of cognitive decline as a continuous measure and longer follow-up period could potentially account for the disparity between the present significant associations and the null findings from this previous study.

The present results also contrast with those from the three previous prospective analyses (all of which were RCTs) of hypoglycaemia and cognitive decline which is short of dementia in people with type 2 diabetes. In ACCORD-MIND and ADVANCE patients in intensive treatment groups (with higher incidence of hypoglycemia) cognitively declined at similar rates over 40-months and five-years of follow-up, respectively, compared with the respective standard treatment groups (de Galan et al., 2009; Launer et al., 2011). However, as noted in Chapter 3, both were RCTs involving strict glycemic control, with cognitive function as a secondary endpoint. Because improving glycemic control may improve cognitive dysfunction when glycemic control is suboptimal (Ryan et al., 2006), detrimental effects of hypoglycemia were potentially counteracted by the specific therapeutic interventions. Future re-analyses of the data from ACCORD-MIND and ADVANCE could apply adjustment for baseline and follow-up HbA1c to evaluate this possibility. ADVANCE also assessed cognitive decline using a screening instrument for dementia, which may be insensitive to subtle cognitive changes (Tombaugh & McIntyre, 1992). Annual incidence of hypoglycemic episodes (defined on the basis of criteria comparable to severe hypoglycemia in the present study) in ADVANCE was low compared with the ET2DS and ACCORD-MIND, because none of its participants received insulin treatment. Finally, the null findings by a small RCT on the effects of antihypertensive medication are questionable on the basis of their use of self-report to measure cognitive function, a short five-month follow-up and the inclusion of participants with a wide age range (McGill et al., 2007).

Neurological damage and persistent processing abnormalities following hypoglycaemia

With the overall inconsistency in the evidence on links between hypoglycaemia and cognitive decline short of dementia, studies assessing the neurological evidence may prove informative. In this section, the literature on associations of hypoglycaemia with cerebral damage is reviewed, before pathophysiological mechanisms potentially underlying any associations of hypoglycaemia with cognitive deficits or neurological damage are described.

Studies of humans

Two reviews of the literature (Auer, 2004; Ryan, 2009) note that evidence of lasting brain damage, such as brain atrophy or lesions (Fujioka et al., 1997; Iino et al., 2000), is typically restricted to hypoglycaemic episodes which result in coma, with null findings reported for instance on associations of SH (not usually involving coma) with EEG abnormalities (Brismar, Hyllienmark, Ekberg, & Johansson, 2002) or grey matter volumes (Musen et al., 2006).

Yet, it may be too soon to discount the potential for neurological damage caused by severe hypoglycaemia without coma. One study of children with type 1 diabetes (aged 7 to 17 years) showed that exposure to incident severe hypoglycaemia during two years of follow-up was associated with a concurrent reduction of white matter in occipital and parietal regions (Perantie et al., 2011). With a similarly 'crucial period' for cognitive development in later life (Biessels et al., 2008), neurological damage due to hypoglycaemia may also be likely with an advanced age of the patient. Indeed, in a sample of middle-aged diabetes patients, a control group showed no cortical atrophy on MRI, whereas 45% of subjects in the group with a history of at least five episodes of hypoglycaemia (which usually remains short of coma) did (Perros, Deary, Sellar, Best, & Frier, 1997). The study was relatively small and failed to establish associations of SH with cognitive function, but was supported by two case reports of middle-aged adults describing EEG abnormalities and cerebral atrophy (Schuler, Petersen, Khalaf, & Kerp, 1989), as well as reductions in white and grey matter volumes and white matter lesions (Kirchhoff et al., 2013) following recurrent episodes of severe hypoglycaemia without coma. Clearly, further brain imaging studies in older patients with diabetes and a history of hypoglycaemia are needed.

Animal studies

Contrasting the mixed evidence from human studies, animal models typically reveal lasting damage to the brain following moderate or severe hypoglycaemia even in absence of coma (which is usually detected by a flat EEG). Recurrent severe episodes have been shown to permanently impair hippocampal synaptic plasticity (Yamada et al., 2004), to cause microglia activation, oxidative injury, cortical neuronal death and impairment in spatial learning and memory in rodents (Won et al., 2012). Even a single hypoglycaemic event without loss of consciousness has been related to cell death in the rodent hippocampus (Ennis, Tran, Seaquist, & Rao, 2008) and cortex, including correlations of the severity of the episode with degree of cell death (Tkacs, Pan, Raghupathi, Dunn-Meynell, & Levin, 2005).

Although animal models offer the ultimate level of experimental control and therefore provide useful supplements to the evidence from studies of humans, it should be noted that a comparison of humans with animals is severely restricted. In absence of coma, hypoglycaemia in humans is unlikely last for hours (as is often the case in animal models) and the long-term effects which human studies are concerned with span months and years rather than days or weeks. Animal models also usually involve developing or adult rodents rather than older animals. The aforementioned evidence from animal studies therefore does not allow inference on hypoglycaemia effects on the human brain. It does, however, at least suggest the *possibility* of severe and particularly recurrent hypoglycaemia causing lasting (potentially subclinical) damage to the brain.

Potential pathophysiological mechanisms and issue of confounding

Potential underlying mechanisms by which hypoglycaemia may contribute to brain damage and resulting cognitive deficits are likely to involve the starvation of neurons of glucose previously demonstrated *in vitro* (Northam & Lin, 2010). Additionally, hypoglycaemia appears to lead to changes in synaptic function and neuronal death due to oxidative stress, as well as an inflammatory response involving the release of endogenous neurotoxins and the accumulation of excitatory amino acids (Fujioka et al., 1997; Iino et al., 2000; Languren, Montiel, Julio-Amilpas, & Massieu, 2013).

Of course, the observational study design of the ET2DS does not allow conclusions with respect to the contribution of such mechanisms to the present findings. It also necessitates the consideration of potential confounding variables. Given that in a number of previous studies of people with type 1 or type 2 diabetes, as well as in the ETDS, hypoglycaemia was associated with symptoms of depression, increased anxiety or lower happiness (Kirchhoff et al., 2013; Shao, Ahmad, Khutoryansky, Aagren, & Bouchard, 2013; Strachan, Deary, Ewing, & Frier, 2000; Wredling, Theorell, Roll, Lins, & Adamson, 1992), a confounding or mediating role of such factors in the present associations of hypoglycaemia with cognitive decline may be plausible. Yet, associations of hypoglycaemia with cognitive decline survived post-hoc adjustment for HADS-D (Feinkohl et al., 2014), showing that the findings reported in this thesis were relatively independent of depression.

Confounding by other clinical parameters or by pre-morbid ability is also possible, but the adjustment of associations of hypoglycaemia with cognitive decline for MHVS, as well as for a range of potential confounders measured at baseline (as presented in this thesis) or at year 4 (Feinkohl et al., 2014) did not greatly alter any of the findings. Although confounding by factors not considered in this thesis is possible, we may be relatively confident that the results presented here are not merely reflections of associations of hypoglycaemia with pre-morbid ability or with vascular risk factors, micro- or macrovascular disease (including stroke) which could themselves either causally influence or act as proxies for late-life cognitive decline.

Potential implications for clinical management of patients

The first part of our findings of a two-fold increased risk of hypoglycaemia in lower-ability individuals implies that in cases where an older patient's cognitive ability is reduced, be it due to early signs of cognitive impairment or lower cognitive reserve, the involvement of their carer in the management of treatment may be advisable.

Although the nature of the study design of the ET2DS does not allow an inference of causality and the findings were only of small to medium effect size (with relatively smaller effect size for associations with late-life cognitive decline compared with estimated lifetime decline), they at least show that a hypoglycaemic event may somewhat impact on a patient's cognitive functions and that their post-event cognitive decline may additionally be accelerated compared with patients who remain free of severe hypoglycaemia. These observations appear to be independent of other clinical covariates. In a clinical setting, a patient's history of hypoglycaemia alone may provide some important information in the ascertainment of their risk of future cognitive decline, although effect sizes were much smaller compared with findings in the cognition-hypoglycaemia direction. If future studies find the association reported here to be causal in nature, it will be necessary to address the effect of strict glycaemic control on cognitive function in the clinical management of older people with type 2 diabetes. The balance of risks for complications associated with hypoglycaemia and hyperglycaemia (including micro- and macrovascular disease and cognitive impairment) are perhaps best evaluated on an individual-patient basis, and under consideration of patients' current cognitive status. Overall, however, I conclude that the education of patients with relatively low cognitive function, or their carers, in terms of diabetes and its treatment (to reduce their risk of future hypoglycaemia) may be of greater importance than the cognitive monitoring of patients who have experienced an episode of SH in the past.

9.5.3 Risk factor associations with cognition identified in multivariable analyses of a wider range of risk factors

The second part of the analyses presented in this thesis was concerned with the comparison of the predictive ability of a wider range of metabolic and vascular risk factors with respect to late-life cognitive function and cognitive change in the ET2DS. Contrasting with the analyses of macrovascular disease and severe hypoglycaemia, no assumptions on potential mechanisms underlying any significant contributions of risk factors to the risk of cognitive decline are made on the basis of these multivariable analyses.

'Optimal' model of cognitive decline and comparison with previous literature on multiple risk factors

Despite a recent systematic review reporting that many of a total of 24 potential clinical, demographic and behavioural risk factors reviewed were, individually, more or less strongly associated with the risk for cognitive decline (Plassman, Williams, Burke, Holsinger, & Benjamin, 2010), the present consideration of a wider range of risk factors and the focus on their relative usefulness in the estimation of risk appears to be fairly unique in the literature. In the stepwise regression analysis of 15 potential vascular and metabolic risk factors, inflammation, packyears, HbA1c but only some of the markers of subclinical macrovascular disease (NT-proBNP, cIMT) were identified as significant predictors of four-year cognitive decline. A number of previous studies focusing on single risk factors had previously shown significant associations of inflammation (Marioni et al., 2011; Yaffe et al., 2003), smoking (Anstey et al., 2007) and hyperglycaemia (Lamport, Lawton, Mansfield, & Dye, 2009; Launer et al., 2011) with cognitive impairment.

Recently, the concept of allostatic load has attracted attention in the field of cognitive ageing. Allostatic load, based on the concept on allostasis – the body's ability to achieve and maintain equilibrium through neuronal, endocrine, cardiovascular responses to environmental stressors (Evans & Schamberg, 2009) – is a summary measure of risk factors. These may include hormone levels, HbA1c, blood pressure, waist-hip ratio, HDL and total cholesterol, cortisol, norepinephrine, epinephrine, dehydroepiandrosterone sulphate (DHEA-S) and inflammatory markers (Karlamangla, Singer, McEwen, Rowe, & Seeman, 2002; McEwen & Seeman, 1999; T. E. Seeman, Singer, Rowe, Horwitz, & McEwen, 1997). For instance, summary scores may be calculated for each individual on the basis of scoring within the distribution of each risk factor (e.g., in tertiles). Considering the aforementioned range of risk factors commonly used to obtain allostatic load summary measures, it is unsurprising that allostatic load has been linked to diabetes (Crews, 2007). Allostatic load summary measures have also been shown to predicted three-year, four-year and seven-year cognitive decline short of dementia in a sample of older adults (Karlamangla et al., 2002; T. E. Seeman, McEwen, Rowe, & Singer, 2001; T. E.

Seeman et al., 1997), consistent with the evidence described in Chapter 2 on individual risk factor associations with cognitive decline. However, the usefulness of such a summary measure, which does not offer any information over and above that from analyses of individual risk factors, may be limited. After all, the observation that a measure of allostatic load predicts cognitive decline is relatively meaningless in terms of potential strategies for intervention, given that it does not reflect at all which of its component risk factors was in fact driving the association. Compared with the alloostatic load approach to the data, the present analysis of a total of 15 risk factors and their association with cognitive decline was certainly more informative, and may be used to inform future observational as well as intervention studies.
Effect sizes of the multivariable analyses in the ET2DS were relatively modest, with risk factors accounting for between 0.6% (for NT-proBNP, over and above age, sex, inflammation, packyears, cIMT and HbA1c) and 2.2% (for inflammation, over and above age and sex) of variance in decline in *g*. The present findings do not allow conclusions with respect to causality, but suggest that the (perhaps combined) measurement of these factors, rather than the measurement of factors such as microvascular disease, symptomatic vascular disease or cholesterol, may be useful in the ascertainment of a patient's risk of cognitive decline.

Some previous studies had investigated smaller groups of selected risk factors in stepwise regression analyses similar to the analyses presented in this thesis, but also appeared to aim to ascertain the underlying causes of associations of single risk factors, such as smoking (Stewart et al., 2006) or hypertension (Starr et al., 1997) rather than to compare potential predictive ability amongst risk factors. One rare previous study on a wider range of risk factors was recently performed on older adults undergoing coronary artery bypass grafting. Education, pre-operative medication, pre-operative cognitive decline and a range of clinical risk factors, including the presence of hypertension, hyperlipidaemia, PAD, history of cerebrovascular disease, cerebral infarct on MRI, three-vessel disease, extracorporeal circulation time and ascending aortic atherosclerosis, were all entered in a stepwise logistic regression model on the risk of post-operative cognitive dysfunction; pre-operative declines, ascending aortic atherosclerosis and cerebral infarction were retained as significant predictors (Otomo, Maekawa, Goto, Baba, & Yoshitake, 2013). Due to the nature of the stepwise modelling process, findings depend strongly on the specific variables entered into the model. A comparison of the present analyses with the aforementioned study is therefore extremely difficult. At least the finding of a contribution by aortic atherosclerosis to the model appears to mirror the inclusion of cIMT in the model of four-year cognitive decline presented in this thesis.

Account of the disparity between findings from univariate analyses and multivariable approach

The fact that a baseline history of stroke, of severe hypoglycaemia and baseline peripheral atherosclerosis (intermittent claudication and/or ABI≤0.90) were not included in the 'optimal' model of four-year cognitive decline should not be seen to oppose or invalidate the significant findings for these risk factors in Chapter 6 (Results II) and Chapter 7 (Results III). As reviewed in Chapter 8, it is in the nature of the 'throw-in-everything' approach of the stepwise modelling procedure to neglect the potentially complex inter-relationships amongst the variables entered into the analysis. For instance, if one potential predictor is highly correlated with another, it is likely that only one will be identified as a significant contributor to the outcome under assessment. The fact that both NT-proBNP and cIMT were included in the model (despite both reflecting subclinical atherosclerosis) may be attributed to relatively weak correlations between the two risk factors (r=0.11). Potential mediation of associations by other risk factors is also not considered in the stepwise procedure. Although not part of the present research question aimed at comparing the predictive ability between risk factors, future studies could now apply formal tests of the complex inter-relationships, including mediatory roles, amongst risk factors in their associations with cognitive decline, for instance using structural equation modelling (SEM). Any of the ten risk factors which were not included in the final 'optimal' model of four-year cognitive decline may in fact be important markers of the risk for cognitive decline and, should future studies reveal causal contributions, may yet constitute useful targets for intervention.

Symptomatic macrovascular disease, for instance, could contribute to cognitive decline through mediation by increases in inflammation, given that atherosclerosis initiates a pro-inflammatory response at vessel walls (Nash, 2005), but could be ‘dropped’ from the regression model which then identifies inflammation but not macrovascular disease as a significant predictor.

Influences from the nature of the stepwise modelling procedure on results are also illustrated by the pattern of findings for waist-hip ratio. The risk factor was not a predictor of four-year cognitive change in the multivariable analyses, despite its inclusion in the cross-sectional model of late-life cognitive function and previous prospective evidence of links of obesity with steeper rates of cognitive decline (Abbatecola et al., 2010; Kilander, Nyman, Boberg, & Lithell, 1997; Profenno, Porsteinsson, & Faraone, 2010). This failure to identify waist-hip ratio as a significant contributor to the model may have been due to correlations with other risk factor measurements. Specifically, any associations of waist-hip ratio with cognitive decline may have been masked by the significant association of the inflammation factor and HbA1c with this outcome in the multivariable model. Fat mass is associated with hyperglycaemia and is also an origin of a range of products, including inflammatory markers, which could mediate a relationship of abdominal obesity with cognitive decline (Sundell, 2005). Waist-hip therefore could therefore have been ‘dropped’ from the model despite a potential role in the determination of a person’s risk of late-life cognitive decline. In future studies, structural equation modelling could be applied to formally test such possible pathways, which in the present data appear to be supported by statistically significant associations of a higher waist-hip ratio with a steeper estimated lifetime cognitive decline and a steeper four-year cognitive decline in the largely unadjusted univariate analyses (Appendix D).

In contrast to waist-hip ratio, the nature of the modelling process does not appear to have contributed to the failure of alcohol consumption (identified as a marker of a higher level of late-life cognitive function and shallower estimated lifetime cognitive change in the present and in previous analyses (Fan, O'Donnell, Singh, Pungan, &

Perlmutter, 2008; Hogenkamp et al., 2014; Townsend, Devore, Kang, & Grodstein, 2009)), to be included in the 'optimal' model of four-year cognitive change. Instead, given that even in the largely unadjusted univariate analyses presented in Appendix D, alcohol was unrelated to this specific outcome, late-life level of alcohol consumption does not appear to be a useful marker of the risk of future cognitive decline.

Account of relatively small effect sizes in statistically significant findings from univariate and multivariable analyses

Although on first inspection, the small effect sizes in multivariable models as well as in the analyses of severe hypoglycaemia and macrovascular disease as individual risk factors may be discouraging and so require further discussion. Even with a total of 15 risk factors considered in these analyses, of which five (in addition to age and sex) were included in the respective final 'best fitting' model of late-life cognitive function, estimated lifetime cognitive change and that of four-year cognitive change were able to explain only 19.9%, 16.1% and 8.6% of variance in the respective outcome. The proportion of variance in the data was even smaller when age was excluded from the models. Age, in fact, was identified as the single most important risk factor predicting an individual's cognitive outcome, which in itself is an important finding, because data on age are free and easily obtainable. Consequently, clinicians should initially consider a patient's age in the estimation of their specific risk of cognitive impairment and presumably already do so routinely on an intuitive basis.

The relatively small effect sizes for individual risk factors other than age found in the multivariable models, as well as in the analysis of severe hypoglycaemia and of macrovascular disease, are likely to simply lie in the nature of research into cognitive ageing and are not at all dissimilar to findings from other research studies of the field, as reviewed in Chapters 2 and 3. An individual's pattern of cognitive change is after all likely to be dependent on a wide range of demographic, psychosocial and clinical risk factors. In the present analyses, a relatively large number but by no means cover all of the risk factors were considered. This is because many potential risk factors were not measured in the ET2DS (e.g., genetic factors; nutritional habits) or may even yet be unknown to the research field. However, even with relatively small effect sizes of associations, the present analyses have identified potentially important risk factors for cognitive decline in patients with type 2 diabetes, and so may be assumed to have advanced the research field.

9.6 Conclusions and future directions

The present analyses have made a contribution to the literature on cognitive impairment in type 2 diabetes. In a relatively unique, large-scale epidemiological study focusing entirely on older people with type 2 diabetes, a wide range of potentially modifiable risk factors, including macrovascular disease and hypoglycaemia, were compared in their associations with late-life cognitive decline short of dementia.

In the first set of analyses, single risk factors were focused on with consideration of a comprehensive list of potential confounders, aimed at the identification and exploration of potential underlying mechanisms. A bidirectional relationship between severe hypoglycaemia and cognitive function and decline was identified, with lower ability at baseline predisposing patients to a two-fold increased risk of incident severe hypoglycaemia. Whereas for both stroke and severe hypoglycaemia, associations with cognitive decline between pre-event estimated level and post-event level compared with their associations with subsequent rates of cognitive decline, the opposite pattern was found for the subclinical markers cIMT, NT-proBNP and ABI. Independent of a wide range of potential confounders, including stroke and socioeconomic status, atherosclerosis of the body, which may remain symptomless, correlated with the risk of cognitive decline. We may speculate about potential correlations with the damage to the cerebral vasculature as the basis for these associations in the present observational data. This implies that patients' risk of cognitive impairment could potentially be reduced through the intensive management of vascular risk factors (as has been suggested in a previous study (Petrova, Prokopenko, Pronina, & Mozheyko, 2010)). The findings further show that the subclinical markers of macrovascular disease could be more useful in the ascertainment of a patient's risk of future cognitive decline compared with symptomatic macrovascular disease.

In the second part of the thesis, the predictive ability for cognitive decline of a total of 15 potentially modifiable risk factors was investigated in a multivariable approach relatively unique to the literature on cognitive ageing. Findings overall suggest that (in addition to subclinical macrovascular disease and severe hypoglycaemia), inflammation, smoking and glycaemic control appear to be useful markers for patients' risk of future cognitive decline. The results warrant replication, but demonstrate that in an attempt to identify patients with type 2 diabetes at risk of cognitive impairment, a routine, combined ascertainment of subclinical macrovascular disease, inflammation, glycaemic control and history of smoking and hypoglycaemia could be advocated. Although amongst the three markers of subclinical macrovascular disease, cIMT appeared to be most strongly related to late-life cognitive decline, systemic atherosclerosis could perhaps be best routinely ascertained by ABI, given the relative ease and low cost of measurement compared with NT-proBNP (measured in plasma) and cIMT (measured by ultrasound).

All analyses presented in this thesis merit replication in the observational analysis of other, large-scale cohorts of older adults with type 2 diabetes. Further epidemiological studies following case-control designs could also compare risk factor associations with cognitive decline between people with type 2 diabetes and non-diabetic older adults. This would help to determine whether or not the findings from the ET2DS are specific to people affected by diabetes, and would provide evidence on the role of the risk factors identified to correlated with cognitive decline in patients with type 2 diabetes in the increased risk of cognitive impairment relative to non-diabetic older adults.

Randomised controlled trials - the 'gold-standard' in epidemiological research - may be used in the investigation of some but by no means all individual risk factors and their relationship with the rate of cognitive decline, because only some may be modified through treatment. This may apply, for instance, to patients' vascular risk or inflammation, which are modifiable through lifestyle intervention as well as pharmaceutical treatment. The key advantage of randomised controlled trials is an ability to infer causality from associations- the key problem of cohort studies such as the ET2DS. However, trials are costly and have sample sizes which are usually much smaller than those of cohort-studies. Due to the requirement of participants to adhere to their respective treatment arm, motivation to participate may lead to samples which are not typical of the target population. Attrition of participants following baseline assessment may also be higher compared with observational cohort studies. Finally, follow-up of randomised controlled trials are also usually shorter than those of observational cohort studies, which may have durations of an entire lifetime (e.g., Lothian Birth Cohort of 1921, Gow et al., 2008), and so may not provide evidence on long-term effects.

The use of randomised controlled settings for the investigation of risk factor associations with cognitive decline may also be unfeasible for many risk factors. For instance, genetic factors may not be altered (although this fact provides an opportunity in itself to investigate risk factor associations with cognition, using the Mendelian randomisation approach). Socioeconomic status, which may be a likely starting point of the path towards late-life disease as well as cognitive impairment, is also extremely difficult to manipulate. This is reflected in the observation that an individual's relative position in society tends to remain relatively stable between birth and older age. Although targeted programs and public health policy may help to work against negative effects of a low socioeconomic status on health, these are close to impossible to conduct in randomized controlled settings. Lifestyle interventions targeting body weight or smoking, too, may be likely to be unsuccessful relative to pharmaceutical interventions which require only the taking of medication once or twice per day.

Although glycaemic control may be modified pharmaceutically, the use of randomised controlled trials may also be inappropriate for the analysis of hypoglycaemia and cognitive decline due to potentially opposing effects of hypoglycaemia and improved glycaemic control on cognitive function. Animal models, however, allow the experimental induction of hypoglycaemia without a change in long-term glycaemic control and so may continue to prove useful in the investigation on hypoglycaemia effects on the brain. Such studies perhaps could be modified to reflect hypoglycaemia occurring in humans more closely than is currently the case, for instance by using older and diabetic animals and by exposing animals to relatively briefer episodes of hypoglycaemia.

The ET2DS itself offers further opportunities for analysis. For the purpose of this thesis, only risk factor data collected at baseline (except severe hypoglycaemia, which was ascertained at baseline and at year 4) were used. A re-analysis of the study with consideration of the year 4 data on subclinical macrovascular disease and incidence of symptomatic macrovascular disease during the study period could now assess the relationship of a *progression* of macrovascular disease with the concurrent change in cognitive function in the sample.

With respect to research of cognitive ageing in general, future studies should aim to apply a multi-wave design, including a serial measurement of cognitive function. This would increase the validity of investigations of longitudinal cognitive change. Studies of birth cohorts ascertaining cognitive ability in childhood or young adulthood may be particularly useful. Based on measured rather than estimated data (as used by the ET2DS), such studies enable an analysis of actual lifecourse changes in cognitive function. As a drawback, however, these (unless sample size is very large so that a diabetic sub-sample could be analysed) do not allow an analysis of a sample consisting exclusively of patients with type 2 diabetes.

The present thesis has demonstrated the usefulness of employing a variety of statistical analyses in cognitive ageing research. The focus on single risk factors with adjustment for potential confounders should continue being used in the attempt to explore potential underlying pathophysiological mechanisms linking individual risk factors with late-life cognitive decline in observational settings. Additionally, future studies should consider a wider range of risk factors in an attempt to identify those which may offer information on patients' risk of future cognitive impairment.

With the problems surrounding the use of stepwise regression procedures in the analysis of a wider range of risk factors particularly in the light of complex inter-relationships amongst risk factors, the use of structural equation modelling, including latent growth curve modelling if multi-wave data on cognitive function is available, could prove useful. Latent growth curve modelling is based on previous knowledge of risk factor associations with cognitive decline and allows the testing of specific hypotheses on relationships, including mediation, amongst risk factors and on their relationships with cognitive change. The approach has recently been applied to the study of contributions by smaller groups of risk factors for cognitive ageing (Gow et al., 2011; Gow et al., 2008). Future studies could now extend this to an investigation of a wider array of risk factors. Such analyses would supplement the present comparison of the relative predictive ability amongst risk factors with respect to the risk of cognitive decline and, once followed up by randomised controlled trials where possible, would ultimately help to identify the driving force(s) underlying the observation that cognitive ability declines with age.

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Appendix A: Dementia in the ET2DS

Table A.1: Data searched to identify participants with dementia following completion of the year 4 clinic

Source	N searched
If patient died prior to follow-up, date of death	All
Report of dementia in communication with patient or relatives	If applicable
Mini-Mental-State Examination (MMSE) score below 24 at baseline	All
Missing MMSE score at baseline	All
ISD hospital discharge code for dementia* between 1981 and 2006	All
Baseline self-reported use of medication for dementia** in baseline questionnaire	All
MMSE<24 at follow-up	All
Missing MMSE score at follow-up	All
ISD hospital discharge code for dementia* between baseline ISD request (2006) and June 2011	All
Self-reported use of medication for dementia** in follow-up questionnaire	All
If died between baseline and follow-up, death code for dementia*	All
Psychiatrist diagnosis of dementia obtained from psychiatry/hospital notes (database at Royal Edinburgh Hospital, 'PiMS')	All
GP cognitive questionnaire sent out	Subjects with MMSE<24 baseline/follow-up and/or MMSE missing at baseline/follow-up and/or participants with ISD dementia code but no other criteria fulfilled, and all non-attenders
GP responded to questionnaire	
GP reports that patient suffers from dementia	
Year of diagnosis if available	
GP reports that patient is on dementia medication**	
Comments by GP on cognitive questionnaire/ in personal communication	

* ICD codes: F00, F01, F02, F03, G30 (codes beginning with FXX or GXX were additionally checked to ensure these were unrelated to dementia). **Donepezil (Aricept), Rivastigmine (Exelon), Galantamine (Reminyl), Memantine (Ebixa)
AD, Alzheimer's disease; VaD, vascular dementia

Table A.2: Summary of information on possible dementia diagnosis

		Baseline				Follow-up						GP			
ID	**Self/ relative report**	**low MMSE**	**missing MMSE**	**ISD**	**self-report meds**	**low MMSE**	**missing MMSE**	**ISD**	**self-report meds**	**death code**	**PiMS**	**report of dementia**	**since**	**on meds**	**GP comment**
0001C						yes									
0017F	dementia										yes				
0037C		yes													
0049F				yes											
0050F												VaD	2010	yes	
0060F			yes												
0061E		yes													
0065E							yes								
0077D			yes			yes									
0079F		yes													
0084B		yes													
0113B						yes									
0152A						yes									
0197D						yes									
0224F		yes													referred to old age psych, probably

															refused
0230B			yes												
0243A	dementia (husband report)														old age psych: cognitive problems due to low mood
0260E															GP phoned summer 2011; in process of diagnosis; unable to contact GP since
0268F	FTD	yes				yes		yes							
0283E		yes													
0299E	research team: possible dementia														
0320C		yes						yes		yes					
0322F	dementia				yes						yes	"senile dementia"	2009	yes	
0342C		yes					yes								
0392B		yes													
0397E		yes													
0423C						yes									
0429E												VaD	2008		
0437C		yes				yes									
0439E	VaD							yes							
0479D		yes													
0487D						yes									
0503D							yes								
0504A							yes								

0506E							yes								old age psych: does not have dementia
0520E		yes						yes			yes				
0537C		yes													
0588C		yes													
0607E		yes													
0615F											yes	VaD	2010		
0634E		yes										VaD	2008		
0663A		yes				yes									
0670A			yes												
0675E						yes									
0692C		yes		yes		yes		yes				VaD	2004		
0715B						yes						mixed AD and VaD	2011		
0718F		yes													
0795D		yes													
0815F		yes													
0824F		yes				yes						mixed AD and VaD	2011	yes	
0838C	dementia							yes				VaD	2008	yes	
0846F						yes									
0850F											yes				
0856F						yes									
0865A												"dementia"	2010		
0868F						yes									
0961B		yes				yes									MCI diagnosis
0970C						yes									
0973F		yes													
1013A	dementia										yes	VaD		yes	
1116A							yes								

1131F							yes								MCI diagnosis
1133B											yes				
1155C	dementia							yes							
1162E		yes													
1166F			yes												
1184C							yes								
1201F		yes													
1212D		yes													
1230F		yes													

Data only reported for subjects who meet at least one of the criteria for dementia and/or failed to attend the year 4 clinic.
'Old age psych', old age psychiatrist; GP, general practitioner; MCI, mild cognitive impairment; FTD, fronto-temporal dementia; VaD, vascular dementia; AD, Alzheimer's Disease; 'meds' or 'GP meds', self-report/ GP report of Donepezil (Aricept), Rivastigmine (Exelon), Galantamine (Reminyl), Memantine (Ebixa) ; ISD, ICD codes: F00, F01, F02, F03, G30 (codes beginning with FXX or GXX were additionally checked to ensure these were unrelated to dementia); 'death code', ICD codes: F00, F01, F02, F03, G30 (codes beginning with FXX or GXX were additionally checked to ensure these were unrelated to dementia) on death certificate; PiMS, psychiatrist diagnosis of dementia obtained from local psychiatry/hospital notes.

Table A.3: Participants with dementia by year 4 (N = 19)

ID number	Year of diagnosis (if available)
0017C	
0050F	2010
0268F	
0320C	
0322F	2009
0429E	
0439E	
0520E	
0615F	2010
0634E	2008
0692C	2004
0715B	2011
0824F	2011
0838C	2008
0850F	
0865A	2010
1013A	
1133B	
1155C	

Patients identified on the basis of information shown in Table A.2

Table A.3: Baseline risk factors according to dementia group

	Dementia (total n=19)		No dementia (total n=1047)		
	Mean, median or N	SD, 95% CI or %	Mean, median or N	SD, 95% CI or %	p-value for trend
Age	71.13	3.40	67.85	4.20	0.001
Male sex	11	57.9	536	51.2	0.56
Education					<0.001
University degree	3	15.8	168	16.0	
Professional qualification	4	21.1	303	28.9	
Secondary school	10	52.6	571	54.5	
Primary school	2	10.5	5	0.5	
Scottish Index of Multiple Deprivation (SIMD) rank					0.43
1st quintile	4	21.1	123	11.7	
2nd quintile	5	26.3	203	19.4	
3rd quintile	1	5.3	187	17.9	
4th quintile	4	21.1	190	18.1	
5th quintile	5	26.3	344	32.9	
HADS-D	5	3 - 11	5	3 - 8	0.005
Duration of diabetes (years)	5	3 - 17	6	3 - 11	0.63
Current treatment					0.76
Insulin +/-tablets	4	21.1	182	17.4	
Tablets alone	13	68.4	666	36.6	
Diet alone	2	10.5	198	18.9	
BMI (kg/m^2)	27.58	4.15	31.50	5.69	0.003
Waist-hip ratio	0.96	0.08	0.97	0.08	0.93
% body fat	35.36	6.91	38.13	7.55	0.12
Total cholesterol (nmol/L)	4.52	1.28	4.31	0.90	0.50
HDL cholesterol (nmol/L)	1.31	0.33	1.29	0.36	0.81
LDL:HDL ratio	3.55	1.10	3.53	1.08	0.94
Systolic bp at clinic visit	136	19	133	16	0.47
Diastolic bp at clinic visit	67	7	69	9	0.41
Historical systolic bp	137	9	138	10	0.66
Ankle brachial index	0.92	0.19	0.96	0.17	0.32
Packyears	9	0 - 30	7	0 - 32	0.59
Alcohol (units/year) groups					0.31
0	7	36.8	291	28.7	
1	3	15.8	184	18.2	
2	4	21.1	222	21.9	
3	5	26.3	167	16.5	
4	0	0	149	14.7	
Cortisol	721.02	212.29	722.21	196.14	0.98
CRP	1.62	0.48 – 3.10	1.86	0.88 – 4.40	0.08
IL-6	3.05	2.27 – 4.85	2.87	1.94 – 4.47	0.47
Fibrinogen	3.48	0.55	3.65	0.75	0.32
TNF-α	1.15	0.46 – 1.44	1.07	0.69 – 1.63	0.26
Inflammation factor	-0.22	0.90	0.00	1.00	0.34
Retinopathy					0.42
no	11	61.1	694	67.6	
mild	7	38.9	285	27.8	

moderate/ severe	0	0.0	47	4.6	
Symptomatic MVD					0.26
MI	6	31.6	144	13.8	
Angina	5	26.3	293	28.0	
Stroke	2	10.5	60	5.7	
TIA	2	10.5	29	2.8	
PAD	0	0.0	65	6.2	
NT-proBNP	107.5	60 - 237	74.5	37 - 169	0.27
Carotid IMT	1.00	0.08	0.99	0.17	0.83
Historical HbA1c (%)	7.26	1.30	7.40	0.89	0.49
HbA1c at clinic visit (%)	7.43	2.19	7.40	1.09	0.95
Severe hypoglycaemia	1	6.3	112	10.9	0.55

CI, confidence interval. Log-transformed values were used for analyses of duration of diabetes, HADS-D, NT-proBNP, CRP, TNF-α and IL-6. Packyears has been square root transformed. These variables are displayed as medians and 95% CI.
N=13 to 19 in ‘dementia’ group; N = 904 to 1047 in ‘no dementia’ group.
MVD, macrovacular disease; carotid IMT, carotid intima-media thickness; NT-proBNP, N-terminal pro-brain natriuretic peptide; ABI, ankle brachial pressure index; MI, myocardial infarction; TIA, transient ischaemic attack; PAD, peripheral arterial disease; CRP, c-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale;

Appendix B: Distribution of variables

Distributions of risk factors at baseline

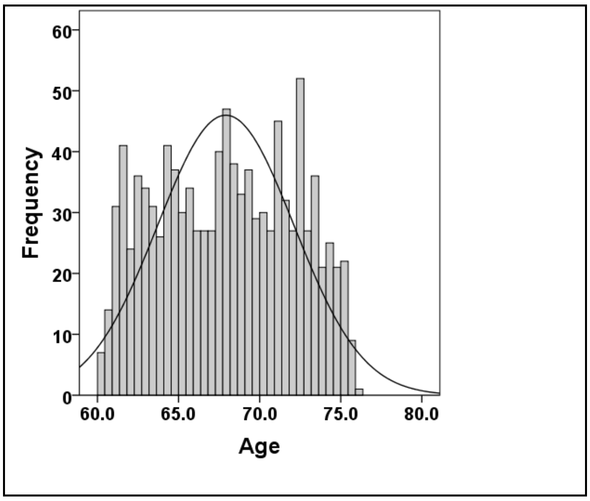


Figure B.1: Distribution of age

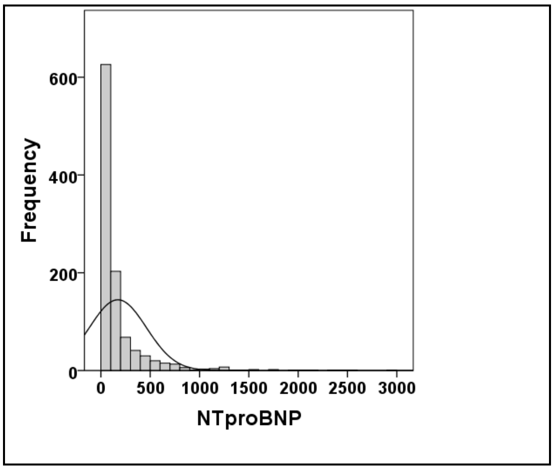


Figure B.2: Distribution of NT-proBNP

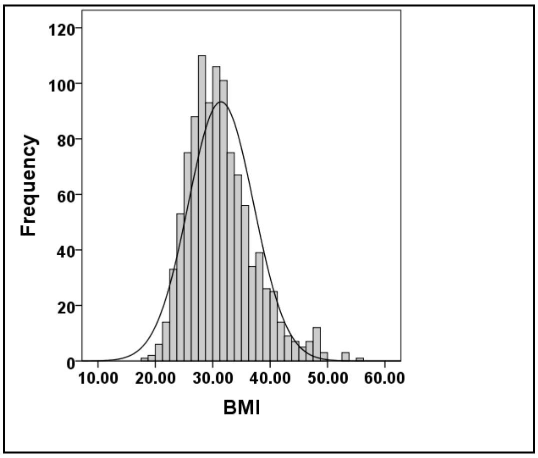


Figure B.3: Distribution of BMI

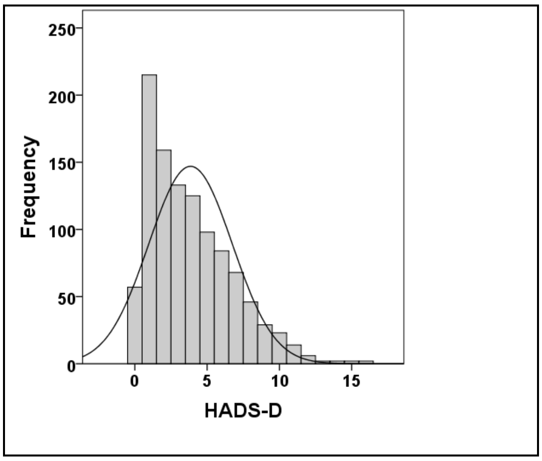


Figure B.4: Distribution of HADS-D score

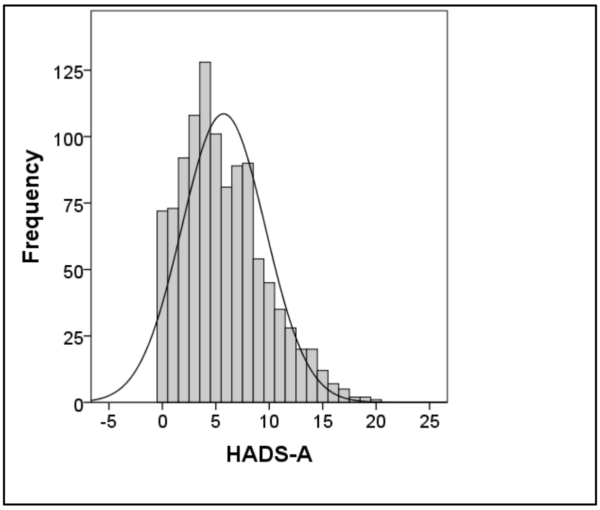


Figure B.5: Distribution of HADS-A score

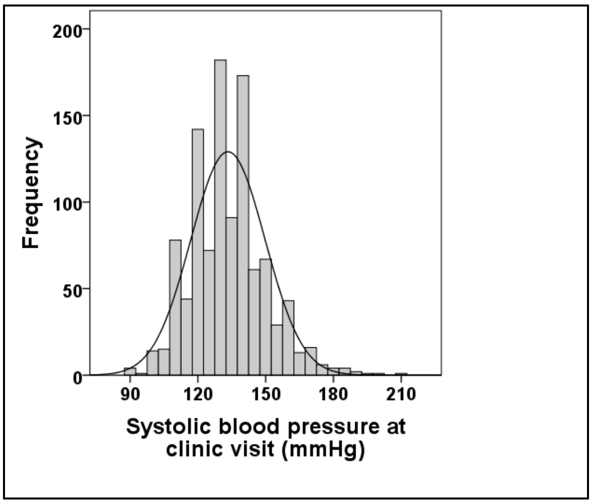


Figure B.6: Distribution of systolic blood pressure at clinic visit

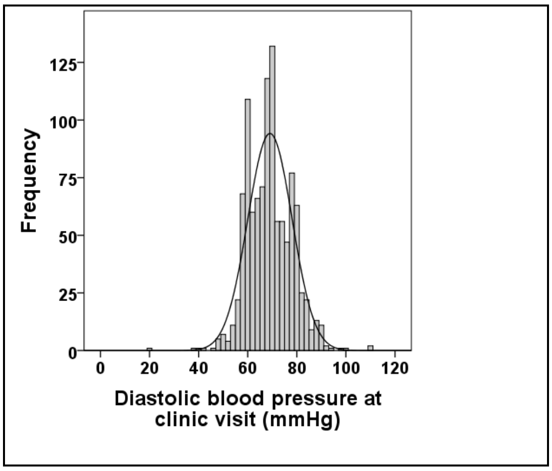


Figure B.7: Distribution of diastolic blood pressure at clinic visit

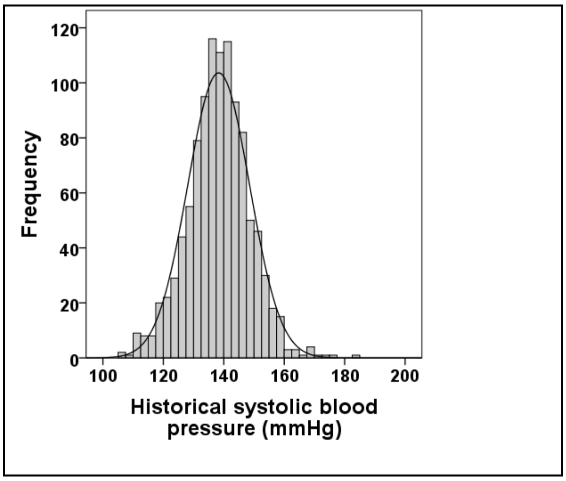


Figure B.8: Distribution of historical systolic blood pressure

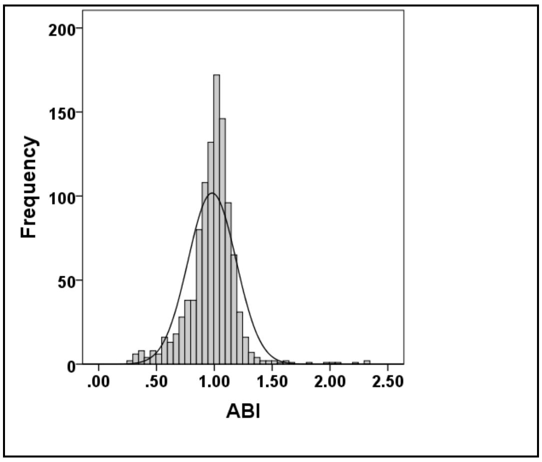


Figure B.9: Distribution of ABI

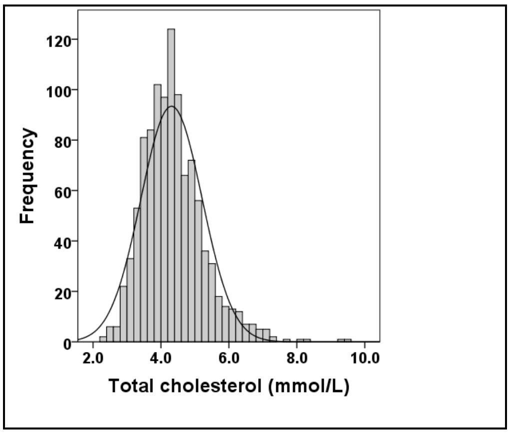


Figure B.10: Distribution of total cholesterol

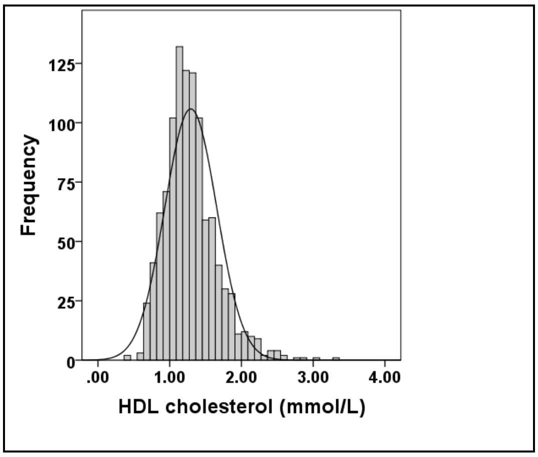


Figure B.11: Distribution of HDL cholesterol

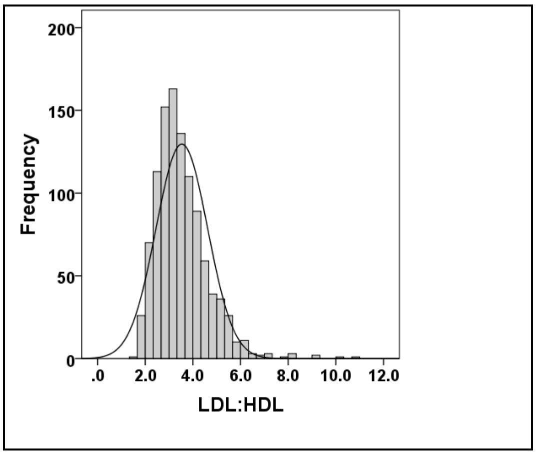


Figure B.12: Distribution of LDL:HDL

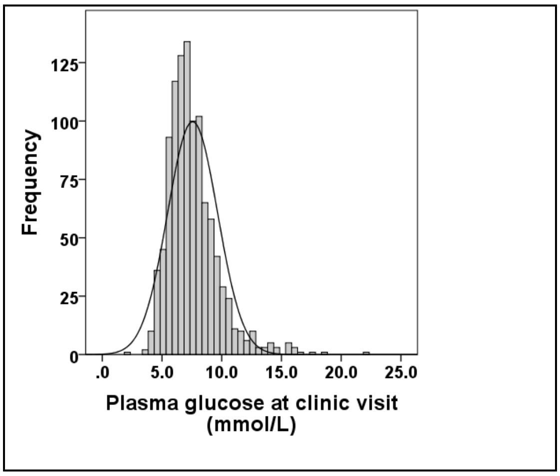


Figure B.13: Distribution of fasting plasma glucose at clinic visit

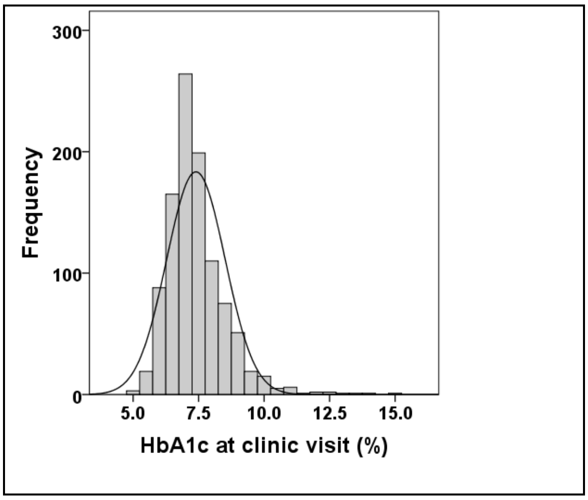


Figure B.14: Distribution of HbA1c at clinic visit

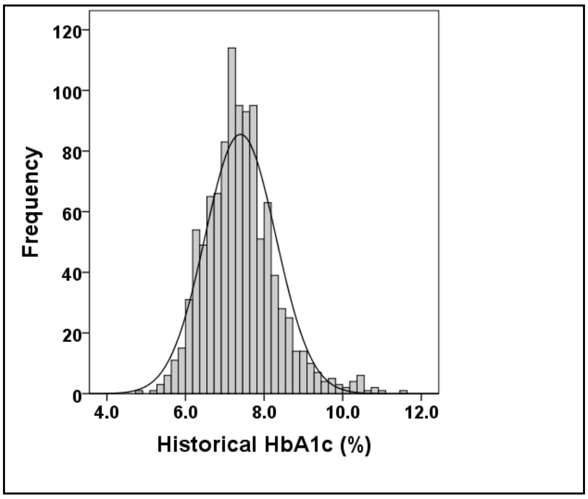


Figure B.15: Distribution of historical HbA1c

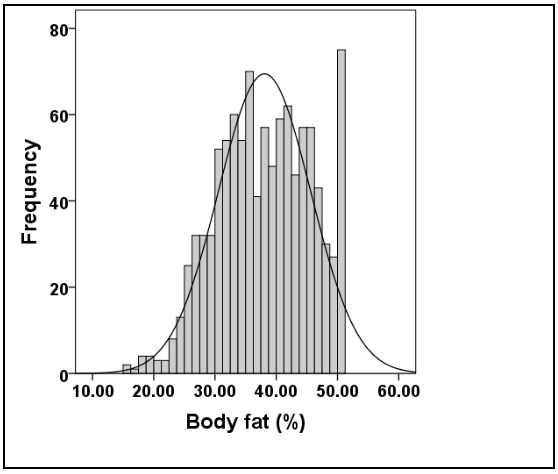


Figure B.16: Distribution of body fat (%)

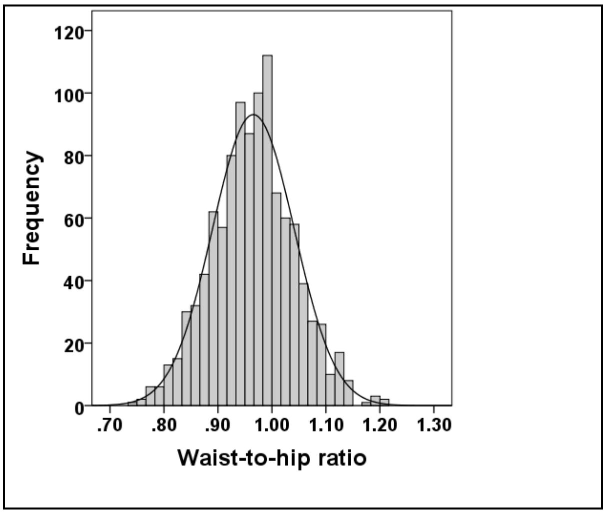


Figure B.17: Distribution of waist-to-hip ratio

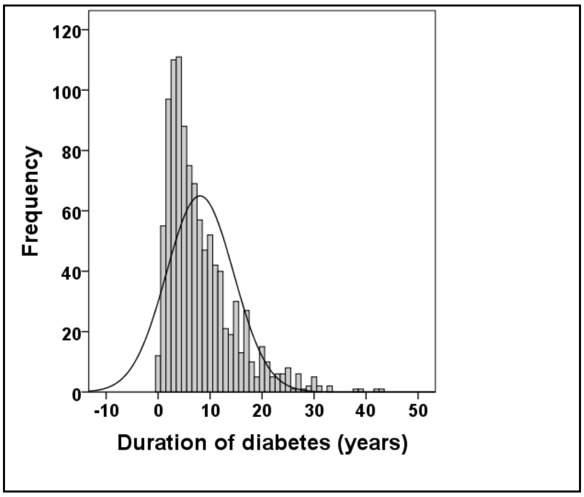


Figure B.18: Distribution of duration of diabetes

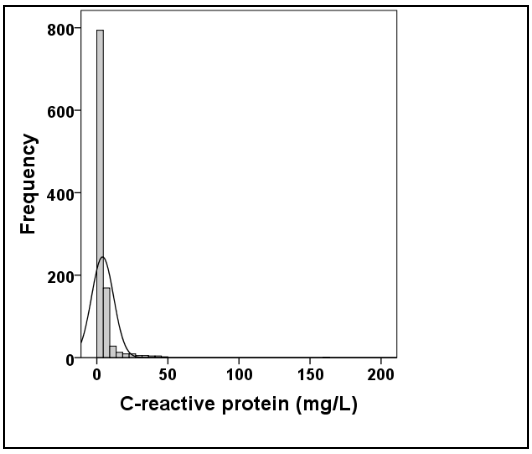


Figure B.19: Distribution of c-reactive protein

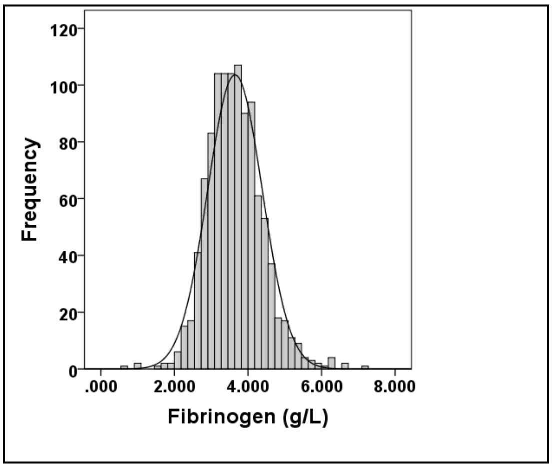


Figure B.20: Distribution of fibrinogen

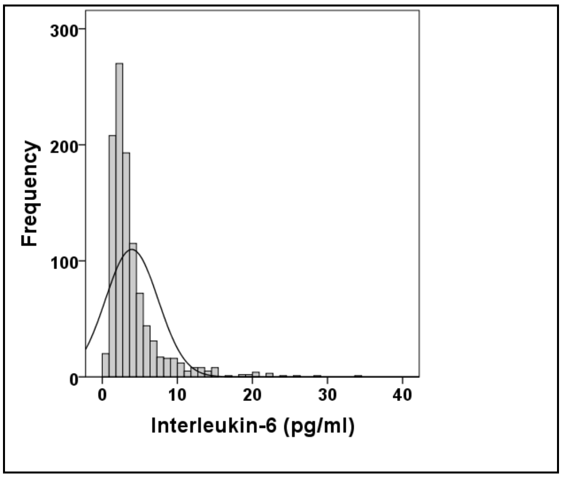


Figure B.21: Distribution of interleukin-6

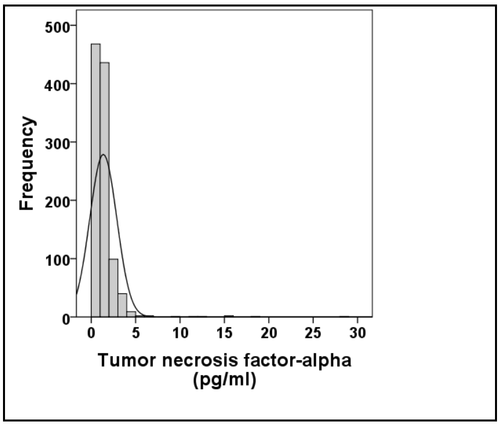


Figure B.22: Distribution of TNF-α

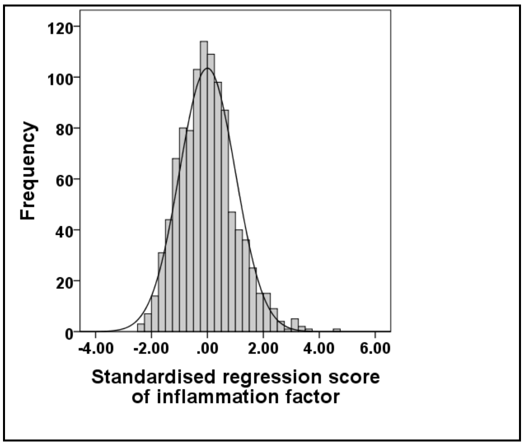


Figure B.23: Distribution of inflammation 'factor'

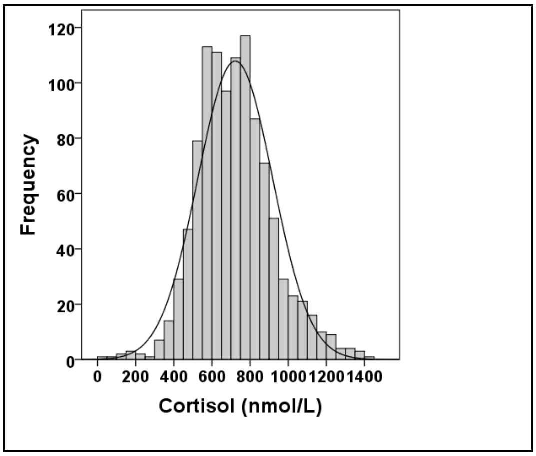


Figure B.24: Distribution of cortisol (nmol/l)

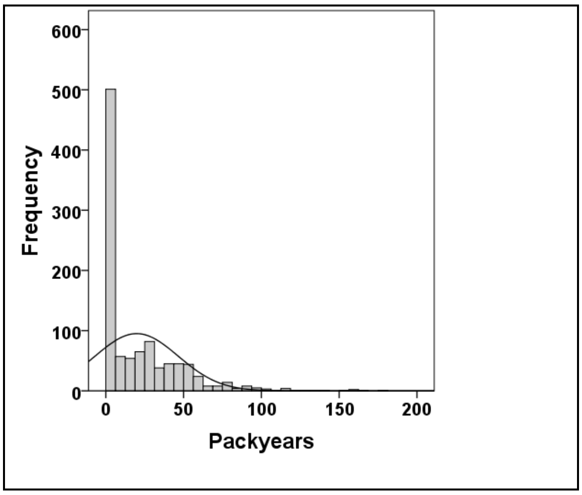


Figure B.25: Distribution of packyears

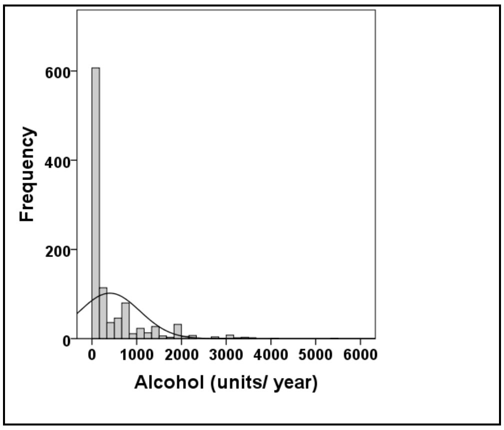


Figure B.26: Distribution of alcohol intake in year prior to baseline clinic

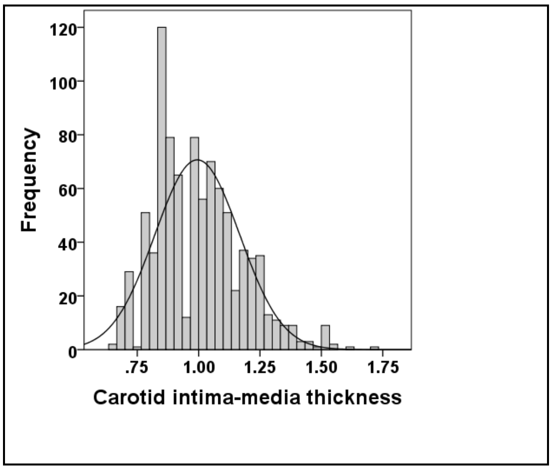


Figure B.27: Distribution of cIMT (measured at Year 1)

Distributions of selected baseline risk factor variables following transformation

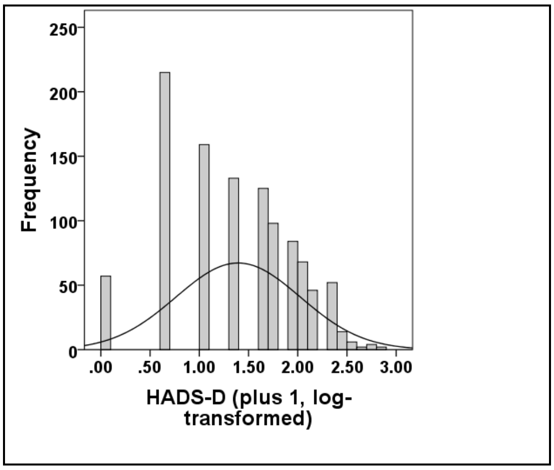


Figure B.28: Distribution of HADS-D score (plus 1) following log-transformation

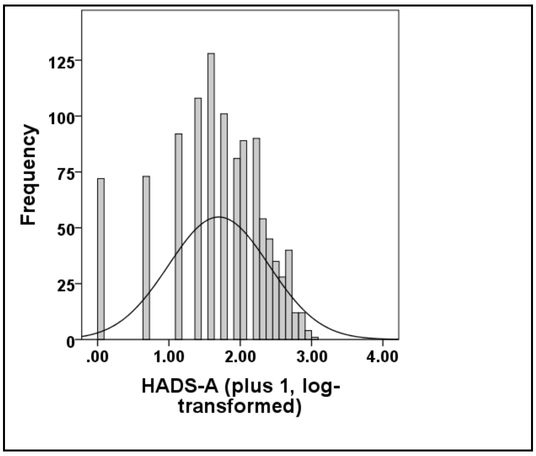


Figure B.29: Distribution of HADS-A score (plus 1) following log-transformation

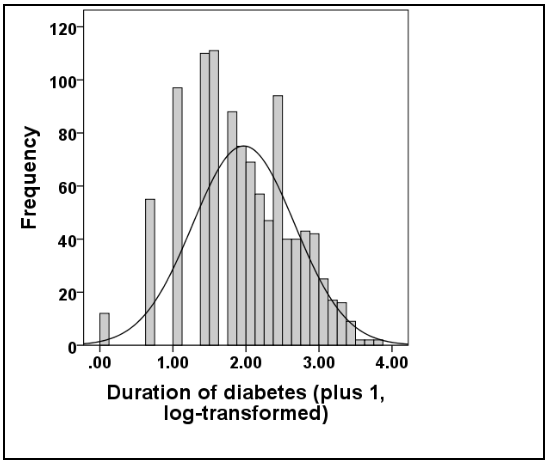


Figure B.30: Distribution of duration of diabetes at baseline (plus 1) following log-transformation

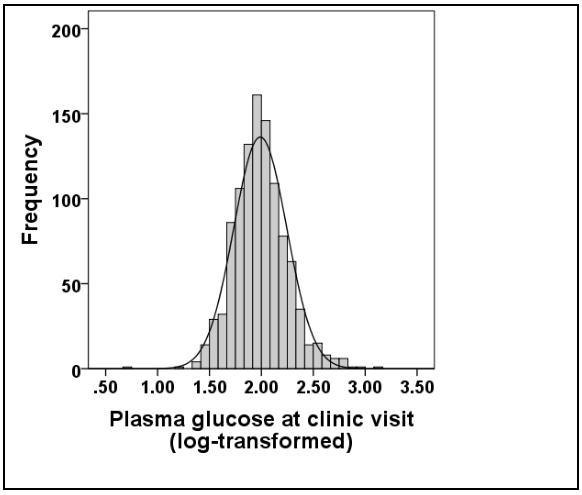


Figure B.31: Distribution of plasma glucose following log-tranformation

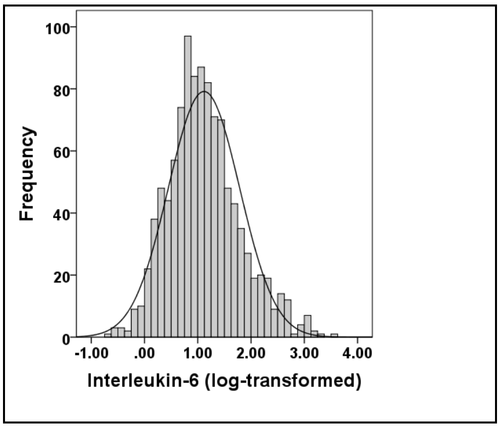


Figure B.32: Distribution of interleukin-6 following log-transformation

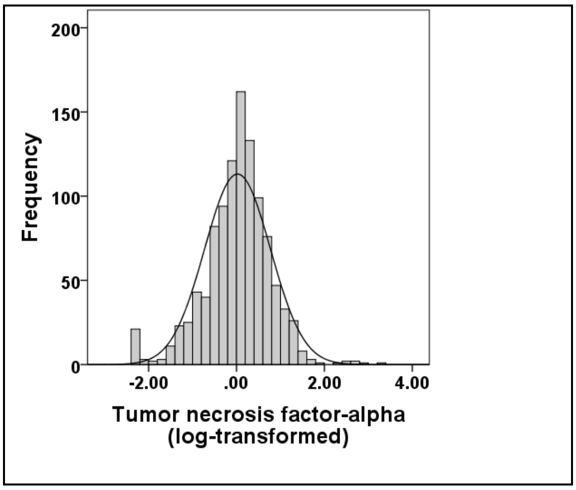
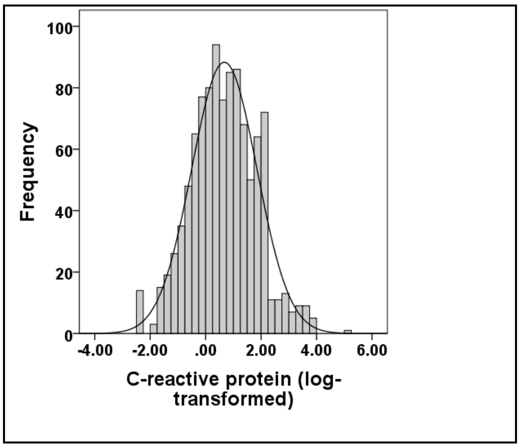
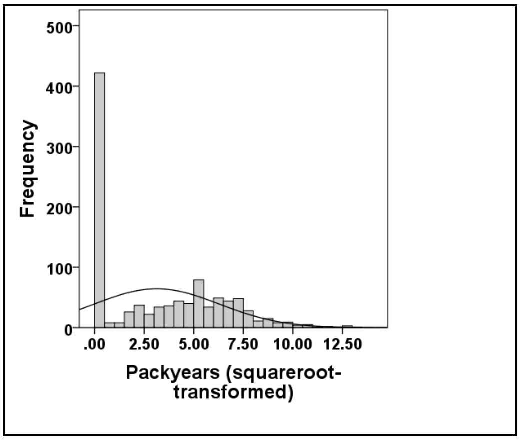


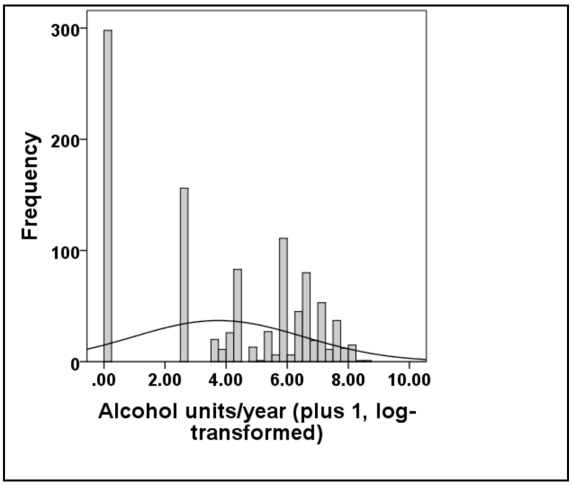
Figure B.33: Distribution of TNF-α following log-transformation



<u>Figure B.34: Distribution of c-reactive protein following log-transformation</u>



<u>Figure B.35: Distribution of packyears plus 1 following square root transformation</u>



<u>Figure B.36: Distribution of alcohol units/year plus 1 following log-transformation</u>

Distributions of cognitive test scores at baseline

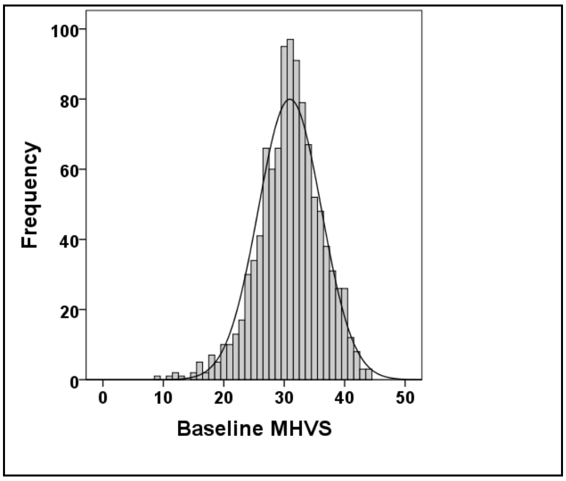


Figure B.37: Baseline distribution of Mill Hill Vocabulary Scale

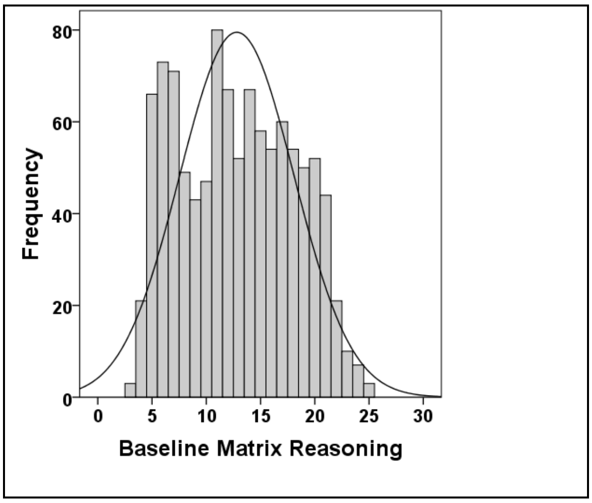


Figure B.38: Baseline distribution of Matrix Reasoning

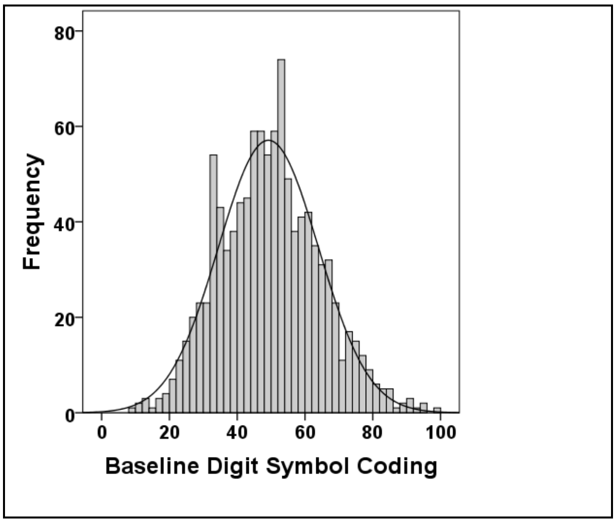


Figure B.39: Baseline distribution of Digit Symbol Coding

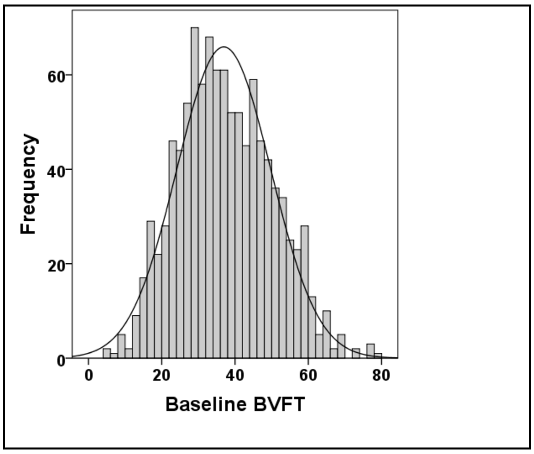


Figure B.40: Baseline distribution of Borkowski Verbal Fluency Test

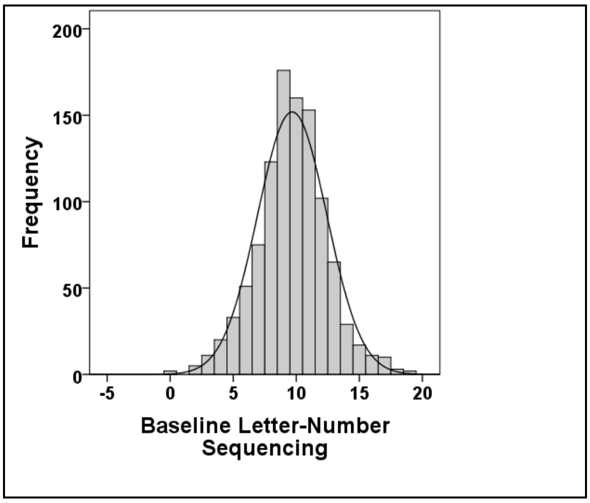


Figure B.41: Baseline distribution of Letter Number Sequencing

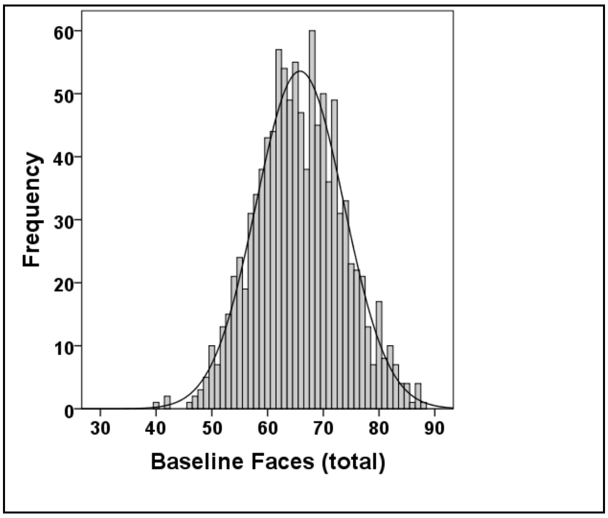


Figure B.42: Baseline distribution of total Faces (immediate and delayed)

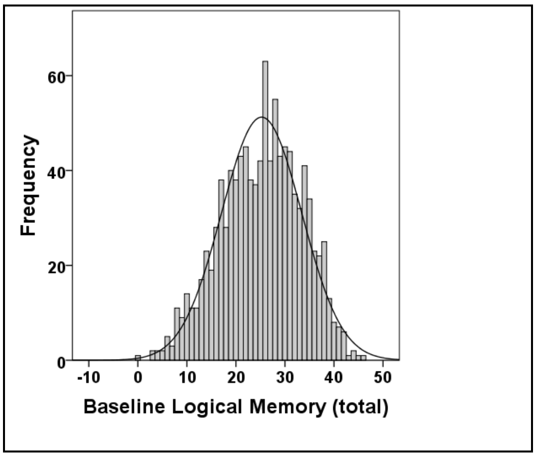


Figure B.43: Baseline distribution of total Logical Memory (immediate and delayed)

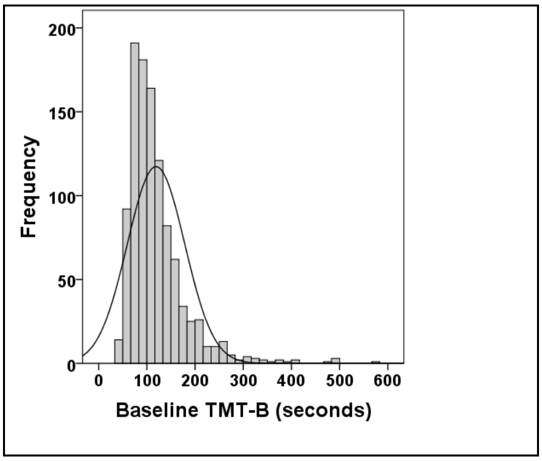


Figure B.44: Baseline distribution of Trail-Making Test B performance (seconds)

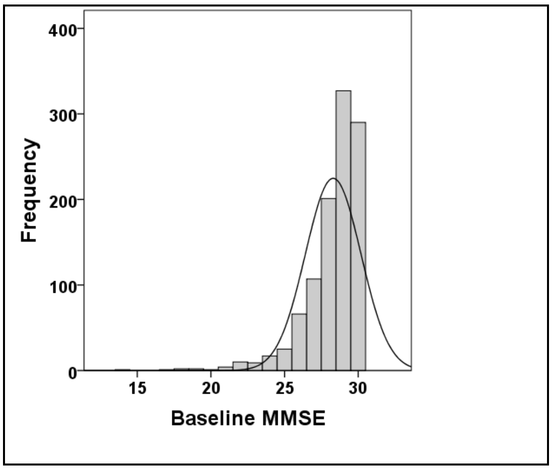


Figure B.45: Baseline distribution of Mini Mental State Examination

Distribution of selected baseline cognitive variables following transformation

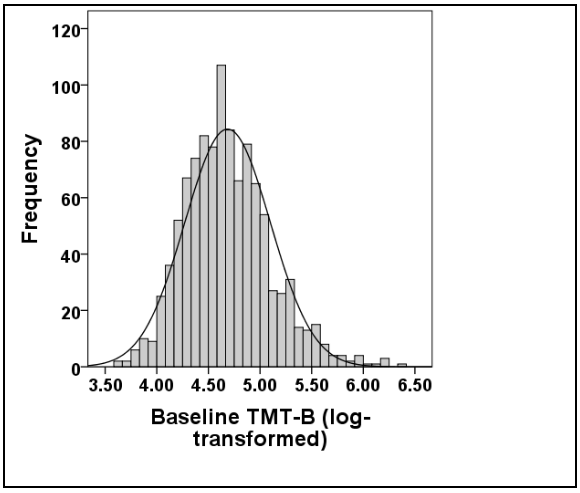


Figure B.46: Distribution of baseline Trail-Making Test-B following log-transformation

Distribution of cognitive test scores at year 4

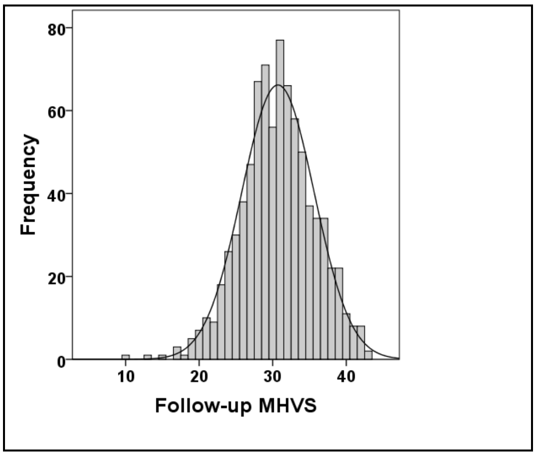


Figure B.47: Follow-up distribution of Mill Hill Vocabulary Scale

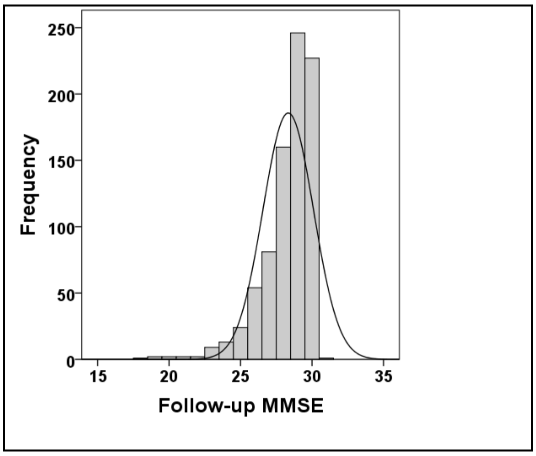


Figure B.48: Follow-up distribution of Mini Mental State Examination

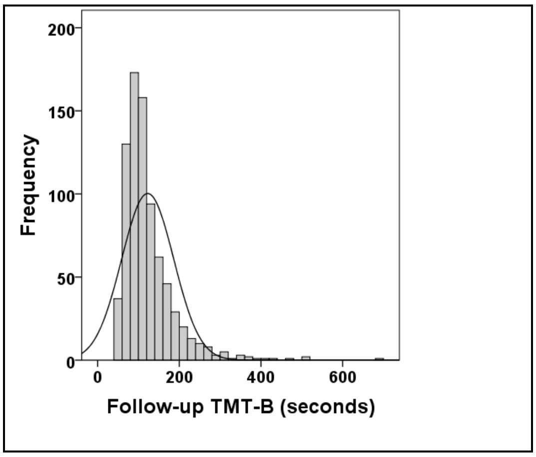


Figure B.49: Follow-up distribution of Trail Making Test-B performance (seconds)

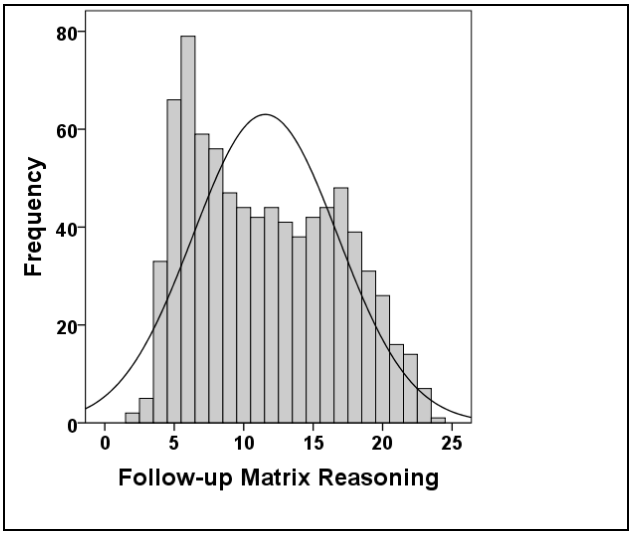


Figure B.50: Follow-up distribution of Matrix Reasoning

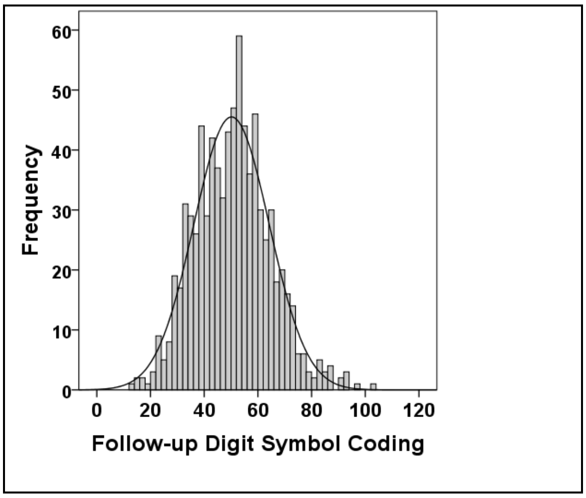


Figure B.51: Follow-up distribution of Digit Symbol Coding

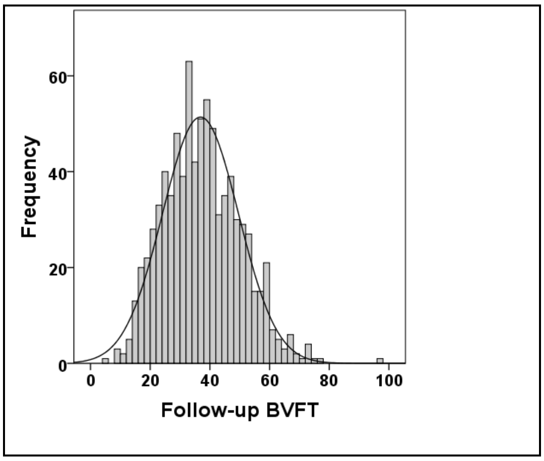


Figure B.52: Follow-up distribution of Borkowski Verbal Fluency

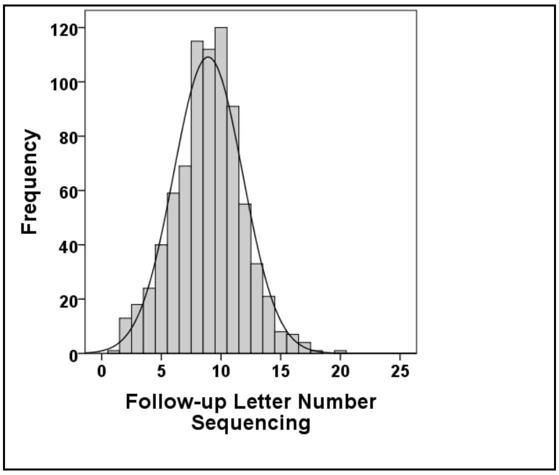


Figure B.53: Follow-up distribution of Letter Number Sequencing

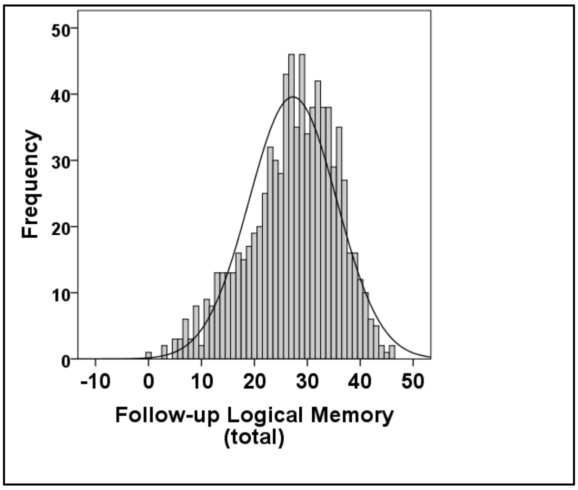


Figure B.54: Follow-up distribution of total Logical Memory (immediate and delayed)

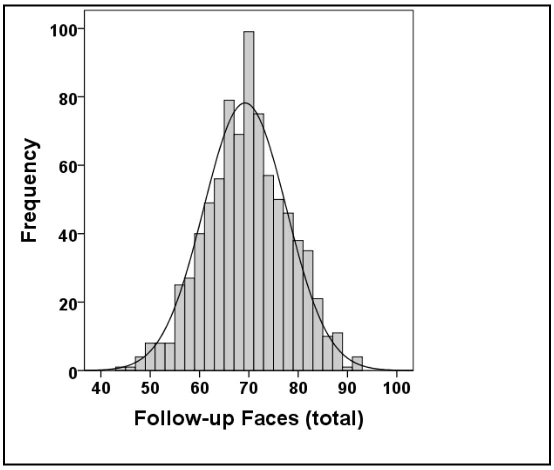


Figure B.55: Follow-up distribution of total Faces (immediate and delayed)

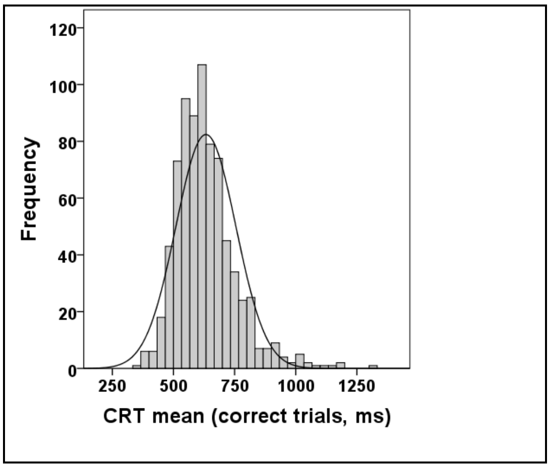


Figure B.56: Follow-up distribution of mean Choice Reaction Time (milliseconds)

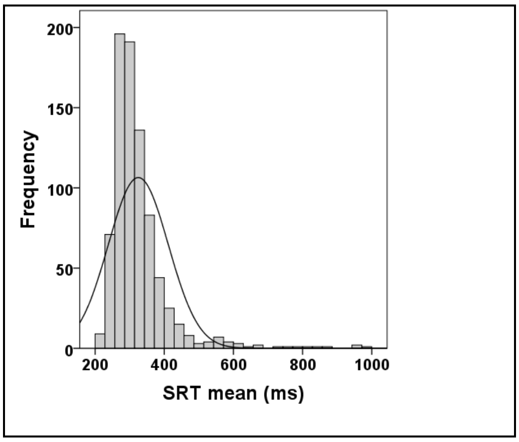


Figure B.57: Follow-up distribution of mean Simple Reaction Time (milliseconds)

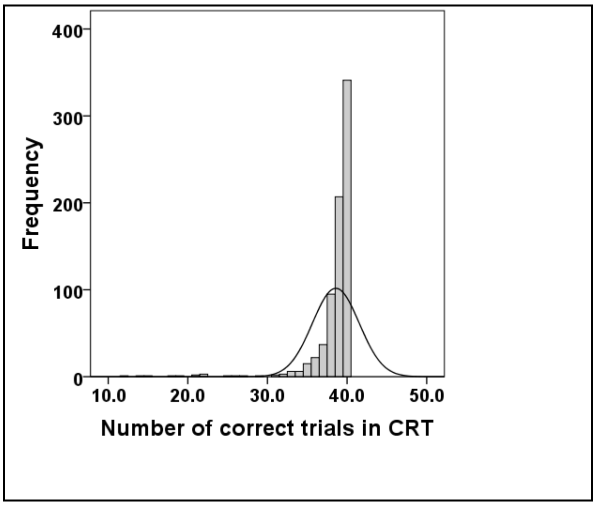


Figure B.58: Number of correct trials in Choice Reaction Time task

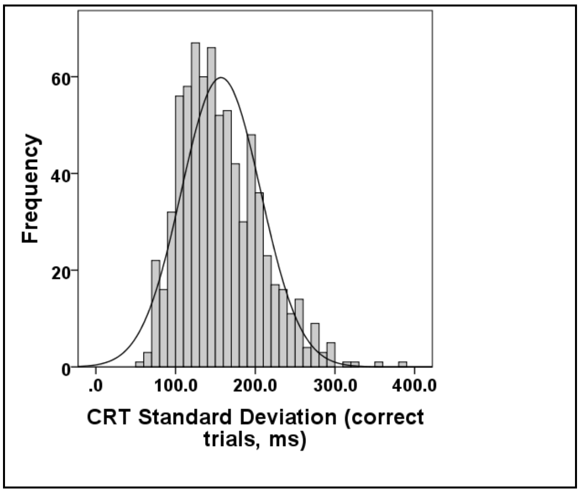


Figure B.59: Standard Deviation in Choice Reaction Time (correct trials)

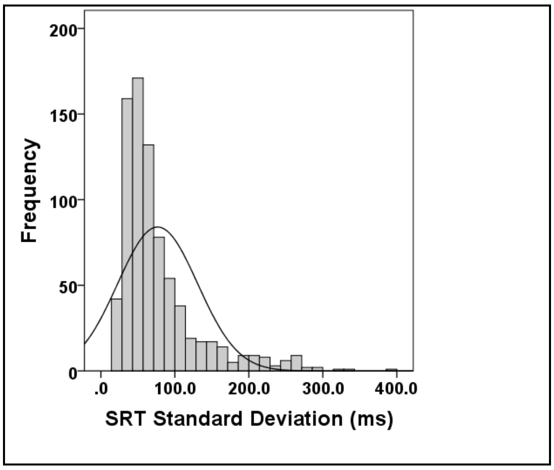


Figure B.60: Standard deviation in Simple Reaction Time (milliseconds)

Distribution of selected year 4 cognitive variables following transformation

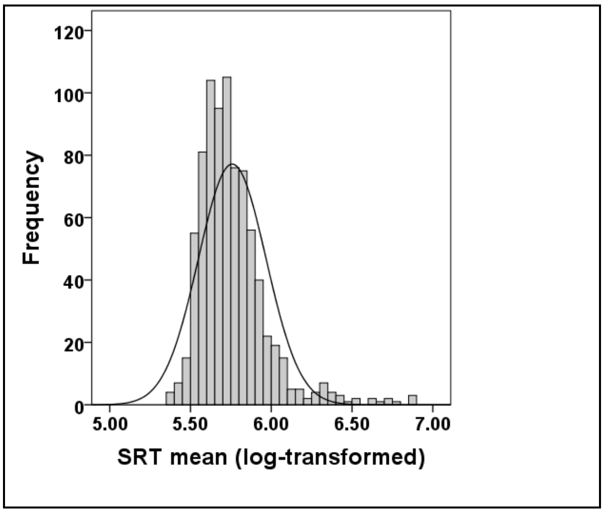


Figure B.61: Distribution of Simple Reaction Time means following log-transformation

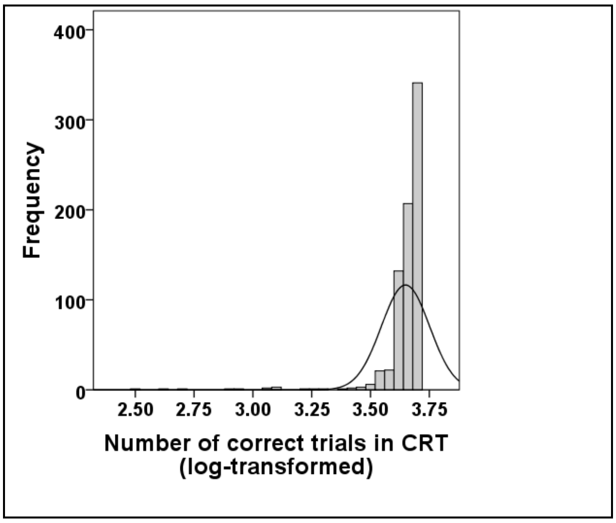


Figure B.62: Number of correct trials in Choice Reaction Time Task following log-transformation

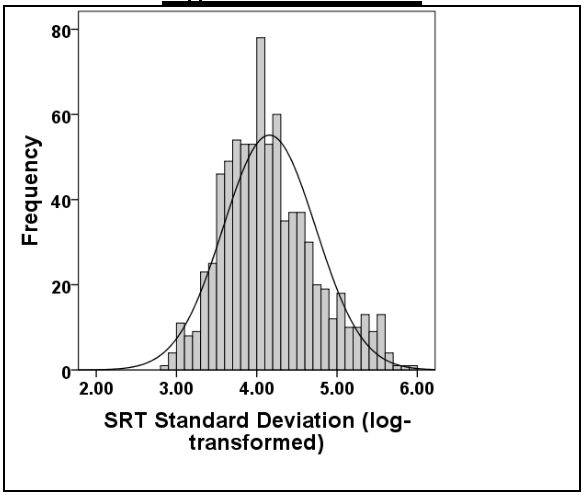


Figure B.63: Standard Deviation in Simple Reaction Time following log-transformation

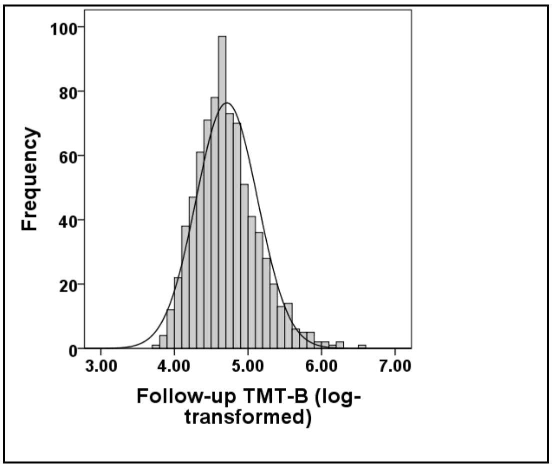


Figure B.64: Distribution of follow-up Trail-Making Test-B following log-transformation

Appendix C: Cognitive change between baseline and year 4 using imputed data for individual cognitive tests

Table C.1: Cognitive change between baseline and year 4 for attenders, using imputed data

		Baseline	Year 4	
	N	**Mean ± SD or median (interquartile range)**	**Mean ± SD or median (interquartile range)**	**p-value**
Logical Memory	824	25.85 ± 7.97	27.27 ± 8.26	<0.001
Faces	824	66.41 ± 7.63	69.25 ± 8.38	<0.001
Matrix Reasoning	825	13.36 ± 5.24	11.55 ± 5.22	<0.001
Digit Symbol Coding	823	50.52 ± 14.30	50.01 ± 14.12	0.150
Trail-Making (s)	823	101 (79 – 133)	107 (83 – 144)	<0.001
Letter-Number Sequencing	823	9.93 ± 2.66	8.86 ± 2.89	<0.001
Verbal Fluency	825	37.83 ± 12.53	36.83 ± 12.75	<0.001

Trail-Making, Trail-Making-Test B; Verbal Fluency, Borkowski Verbal Fluency Test; s, seconds.
Log-transformed values were used for analyses of Trail-Making.

Appendix D: Univariate associations of risk factors with cognitive function

Risk factors and estimated pre-morbid cognitive ability

Table D.1: Univariate association of risk factors with baseline MHVS

	MHVS
Any symptomatic MVD	**-0.14 (<0.001)**
Transient ischaemic attack	-0.01 (0.85)
Stroke	**-0.06 (0.042)**
Myocardial infarction	**-0.06 (0.053)**
Angina	**-0.11 (<0.001)**
Peripheral arterial disease	-0.01 (0.80)
Waist-hip ratio	**-0.16 (<0.001)**
Total cholesterol	0.05 (0.16)
HDL cholesterol	**0.09 (0.004)**
LDL:HDL	-0.04 (0.20)
Systolic bp at clinic visit	0.00 (0.95)
Diastolic bp at clinic visit	0.07 (0.07)
Historical systolic bp	0.03 (0.41)
Packyears	**-0.07 (0.026)**
Alcohol units/year	**0.16 (<0.001)**
ABI	**0.09 (0.006)**
Carotid IMT	0.02 (0.55)
NT-proBNP	-0.06 (0.06)
Inflammation factor	0.06 (0.06)
HbA1c at clinic visit	**-0.06 (0.053)**
Historical HbA1c	-0.03 (0.32)
Cortisol	0.04 (0.20)
Diabetic retinopathy	0.01 (0.73)
Severe hypoglycaemia	-0.05 (0.13)
Duration of diabetes	-0.02 (0.50)
HADS-D	**-0.12 (<0.001)**

Analyses were linear regression analyses adjusted for age and sex. β values are standardised regression correlation coefficients (p-values). Log-transformed variables were used for NT-proBNP, HADS-D, alcohol untis/year and duration of diabetes. Square root transformed values were used for packyears. Alcohol units/year is based on quintiles.
N = 909 to 1049.
Peripheral arterial disease measured by presence of intermittent claudication; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale.

Risk factors and cognitive function at year 4

Table D.2: Univariate association of risk factors with cognitive function at year 4

	'g'	Logical Memory	Faces	Matrix Reasoning	Trail-Making	Digit Symbol Coding	Letter-Number Sequencing	Verbal Fluency
Any symptomatic MVD	**-0.17 (<0.001)**	**-0.12 (0.001)**	**-0.11 (0.002)**	**-0.11 (0.002)**	**0.11 (0.001)**	**-0.15 (<0.001)**	**-0.11 (0.002)**	**-0.11 (0.002)**
Transient ischaemic attack	-0.04 (0.29)	0.01 (0.75)	-0.04 (0.25)	**-0.11 (0.002)**	0.01 (0.68)	-0.02 (0.59)	-0.03 (0.38)	0.01 (0.75)
Stroke	**-0.16 (<0.001)**	**-0.07 (0.040)**	-0.06 (0.11)	-0.06 (0.06)	**0.14 (<0.001)**	**-0.15 (<0.001)**	**-0.07 (0.051)**	**-0.11 (0.002)**
Myocardial infarction	**0.09 (0.013)**	-0.02 (0.50)	-0.04 (0.27)	**-0.10 (0.005)**	0.07 (0.06)	**-0.09 (0.013)**	-0.07 (0.06)	-0.02 (0.50)
Angina	**-0.11 (0.002)**	-0.09 (0.010)	-0.05 (0.17)	**-0.07 (0.041)**	**0.07 (0.053)**	**-0.13 (<0.001)**	**-0.08 (0.020)**	-0.04 (0.27)
Peripheral arterial disease	-0.06 (0.10)	-0.05 (0.18)	-0.06 (0.07)	0.00 (0.97)	0.05 (0.13)	-0.01 (0.70)	-0.06 (0.11)	-0.04 (0.21)
Waist-hip ratio	**-0.20 (<0.001)**	-0.06 (0.15)	-0.06 (0.14)	**-0.13 (0.001)**	**0.18 (<0.001)**	**-0.21 (<0.001)**	**-0.15 (<0.001)**	**-0.14 (0.001)**
Total cholesterol	0.00 (0.92)	-0.05 (0.19)	0.03 (0.46)	0.02 (0.49)	0.00 (0.96)	-0.02 (0.51)	0.03 (0.47)	-0.03 (0.42)
HDL cholesterol	**0.09 (0.014)**	0.06 (0.12)	**0.07 (0.036)**	**0.07 (0.049)**	-0.05 (0.18)	0.03 (0.40)	**0.08 (0.036)**	**0.09 (0.015)**
LDL:HDL	**-0.08 (0.022)**	**-0.08 (0.18)**	-0.02 (0.48)	-0.06 (0.10)	0.04 (0.24)	-0.06 (0.12)	-0.04 (0.28)	**-0.10 (0.006)**
Systolic bp at clinic visit	-0.06 (0.10)	-0.03 (0.43)	-0.03 (0.33)	-0.04 (0.21)	0.03 (0.40)	-0.01 (0.82)	**-0.08 (0.018)**	**-0.07 (0.035)**
Diastolic bp at clinic visit	0.01 (0.79)	0.00 (0.99)	0.00 (0.98)	0.02 (0.66)	-0.01 (0.75)	0.03 (0.48)	-0.02 (0.58)	0.00 (0.97)
Historical systolic bp	0.02 (0.48)	0.03 (0.46)	-0.02 (0.57)	0.00 (0.90)	-0.02 (0.63)	0.03 (0.47)	0.03 (0.43)	0.01 (0.74)
Packyears	**-0.15 (<0.001)**	-0.05 (0.19)	**-0.14 (<0.001)**	**-0.12 (0.001)**	**0.11 (0.003)**	**-0.17 (<0.001)**	**-0.07 (0.048)**	-0.05 (0.17)
Alcohol units/year	**0.18 (<0.001)**	**0.13 (0.001)**	**0.10 (0.007)**	**0.13 (0.001)**	**-0.11 (0.003)**	**0.09 (0.019)**	**0.13 (0.001)**	**0.17 (<0.001)**
ABI	**0.12 (0.001)**	**0.11 (0.003)**	**0.07 (0.041)**	0.06 (0.09)	**-0.10 (0.004)**	**0.13 (<0.001)**	0.05 (0.14)	0.02 (0.51)
Carotid IMT	**-0.09 (0.014)**	-0.05 (0.16)	**-0.09 (0.014)**	-0.09 (0.010)	0.06 (0.09)	-0.05 (0.19)	-0.01 (0.88)	-0.04 (0.28)
NT-proBNP	**-0.10 (0.005)**	**-0.09 (0.014)**	-0.07 (0.06)	-0.04 (0.21)	0.07 (0.06)	**-0.07 (0.038)**	**-0.07 (0.048)**	-0.06 (0.08)
Inflammation factor	**-0.20 (<0.001)**	**-0.08 (0.026)**	**-0.11 (0.002)**	**-0.12 (0.001)**	**0.15 (<0.001)**	**-0.17 (<0.001)**	**-0.16 (<0.001)**	**-0.11 (0.003)**
HbA1c at clinic visit	**-0.12 (0.001)**	-0.01 (0.70)	**-0.08 (0.022)**	**-0.08 (0.022)**	**0.09 (0.013)**	**-0.09 (0.012)**	**-0.08 (0.019)**	**-0.15 (<0.001)**
Historical HbA1c	**-0.12 (0.001)**	0.02 (0.50)	**-0.10 (0.002)**	-0.06 (0.06)	**0.11 (0.001)**	**-0.09 (0.013)**	**-0.07 (0.051)**	**-0.13 (<0.001)**
Cortisol	0.00 (0.98)	-0.03 (0.42)	-0.02 (0.62)	0.02 (0.56)	0.01 (0.76)	0.03 (0.36)	-0.01 (0.83)	-0.01 (0.74)

Diabetic retinopathy	-0.05 (0.17)	-0.04 (0.27)	-0.02 (0.54)	-0.01 (0.71)	0.05 (0.19)	-0.03 (0.36)	-0.03 (0.48)	-0.04 (0.24)
Severe hypoglycaemia	**-0.09 (0.009)**	0.02 (0.66)	-0.02 (0.52)	**-0.09 (0.009)**	**0.11 (0.001)**	**-0.09 (0.009)**	-0.06 (0.10)	-0.06 (0.10)
Duration of diabetes	**-0.09 (0.006)**	-0.02 (0.52)	**-0.09 (0.008)**	-0.06 (0.09)	**0.07 (0.045)**	**-0.08 (0.020)**	-0.06 (0.12)	**-0.09 (0.010)**
HADS-D	**-0.20 (<0.001)**	**-0.10 (0.004)**	-0.06 (0.07)	**-0.12 (0.001)**	**0.17 (<0.001)**	**-0.19 (<0.001)**	**-0.17 (<0.001)**	**-0.14 (<0.001)**

Analyses were linear regression analyses adjusted for age and sex. β values are standardised regression correlation coefficients (p-values). Data for *g* have been imputed, for individual cognitive tests are non-imputed. Log-transformed variables were used for Trail-Making, HADS-D, NT-proBNP and duration of diabetes. Square root transformed values were used for packyears. Alcohol units/year is based on quintiles. N = 739 to 824. Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test.

Table D.3: Univariate association of risk factors with reaction time at year 4

	Simple Reaction Time mean	**Choice Reaction Time mean**
Any symptomatic MVD	0.06 (0.11)	0.06 (0.09)
Transient ischaemic attack	0.01 (0.80)	0.00 (0.94)
Stroke	**0.09 (0.011)**	0.05 (0.19)
Myocardial infarction	-0.03 (0.48)	-0.02 (0.55)
Angina	0.04 (0.23)	0.07 (0.08)
Peripheral arterial disease	-0.01 (0.88)	**0.08 (0.020)**
Waist-hip ratio	**0.13 (0.001)**	**0.18 (<0.001)**
Total cholesterol	-0.03 (0.46)	-0.04 (0.34)
HDL cholesterol	-0.05 (0.16)	-0.06 (0.15)
LDL:HDL	0.03 (0.48)	0.04 (0.23)
Systolic bp at clinic visit	**0.07 (0.050)**	**0.09 (0.010)**
Diastolic bp at clinic visit	-0.03 (0.45)	-0.04 (0.33)
Historical systolic bp	0.02 (0.56)	0.05 (0.21)
Packyears	0.02 (0.69)	**0.08 (0.050)**
Alcohol units/year	-0.02 (0.70)	-0.01 (0.82)
ABI	-0.06 (0.11)	**-0.08 (0.034)**
Carotid IMT	**0.10 (0.005)**	**0.12 (0.002)**
NT-proBNP	0.00 (0.92)	0.04 (0.31)
Inflammation factor	**0.08 (0.026)**	**0.08 (0.025)**
HbA1c at clinic visit	0.04 (0.22)	0.04 (0.26)
Historical HbA1c	**0.08 (0.020)**	0.06 (0.09)
Cortisol	-0.05 (0.16)	-0.03 (0.46)
Diabetic retinopathy	-0.01 (0.70)	0.01 (0.73)
Severe hypoglycaemia	**0.10 (0.007)**	**0.08 (0.031)**
Duration of diabetes	0.04 (0.31)	0.03 (0.36)
HADS-D	0.02 (0.50)	**0.08 (0.020)**

Analyses were linear regression analyses adjusted for age and sex. β values are standardised regression correlation coefficients (p-values). Log-transformed variables were used for SRT mean, HADS-D, NT-proBNP and duration of diabetes . Square root transformed values were used for packyears. Alcohol units/year is based on quintiles. N = 744 to 797 for SRT and 739 to 748 for CRT. Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale. SRT, simple reaction time; CRT, choice reaction time.

Table D.4: Univariate associations of risk factors with cognitive function at year 4 adjusted for MHVS

	'g'	Logical Memory	Faces	Matrix Reasoning	Trail-Making	Digit Symbol Coding	Letter-Number Sequencing	Verbal Fluency
Any symptomatic MVD	**-0.06 (0.030)**	-0.03 (0.31)	-0.05 (0.12)	-0.02 (0.51)	0.05 (0.17)	**-0.08 (0.017)**	-0.03 (0.42)	-0.03 (0.33)
Transient ischaemic attack	-0.02 (0.58)	0.03 (0.29)	-0.04 (0.28)	**-0.08 (0.009)**	0.00 (0.98)	0.00 (0.97)	-0.01 (0.74)	0.02 (0.64)
Stroke	**-0.12 (<0.001)**	-0.04 (0.17)	-0.04 (0.28)	-0.03 (0.30)	**0.12 (<0.001)**	**-0.13 (<0.001)**	-0.05 (0.14)	**-0.08 (0.012)**
Myocardial infarction	-0.05 (0.11)	0.00 (0.95)	-0.02 (0.58)	**-0.08 (0.015)**	0.04 (0.27)	-0.05 (0.11)	-0.04 (0.18)	0.01 (0.81)
Angina	-0.01 (0.73)	-0.02 (0.65)	0.00 (0.95)	0.01 (0.80)	0.00 (0.97)	**-0.07 (0.034)**	0.01 (0.80)	0.03 (0.35)
Peripheral arterial disease	-0.03 (0.26)	-0.03 (0.33)	-0.06 (0.08)	0.01 (0.66)	0.03 (0.29)	0.00 (0.89)	-0.03 (0.42)	-0.03 (0.36)
Waist-hip ratio	**-0.11 (0.001)**	0.02 (0.61)	-0.01 (0.82)	-0.06 (0.07)	**0.13 (0.001)**	**-0.15 (<0.001)**	**-0.09 (0.022)**	-0.07 (0.08)
Total cholesterol	-0.01 (0.71)	-0.05 (0.11)	0.02 (0.63)	0.01 (0.72)	0.00 (0.95)	-0.03 (0.36)	0.03 (0.38)	-0.04 (0.28)
HDL cholesterol	0.03 (0.24)	0.01 (0.79)	0.04 (0.20)	0.03 (0.42)	-0.02 (0.57)	0.00 (0.93)	0.04 (0.27)	0.05 (0.17)
LDL:HDL	-0.05 (0.08)	-0.06 (0.07)	-0.01 (0.77)	-0.04 (0.22)	0.03 (0.45)	-0.04 (0.24)	-0.01 (0.67)	**-0.08 (0.026)**
Systolic bp at clinic visit	-0.05 (0.11)	-0.02 (0.53)	-0.03 (0.38)	-0.04 (0.23)	0.02 (0.47)	0.00 (0.99)	**-0.07 (0.025)**	**-0.07 (0.027)**
Diastolic bp at clinic visit	-0.03 (0.35)	-0.03 (0.39)	-0.02 (0.65)	-0.02 (0.64)	0.02 (0.65)	0.00 (0.99)	-0.04 (0.20)	-0.03 (0.36)
Historical Systolic bp	0.01 (0.76)	0.02 (0.64)	-0.03 (0.42)	-0.02 (0.62)	-0.01 (0.79)	0.01 (0.70)	0.02 (0.58)	0.00 (0.91)
Packyears	**-0.10 (0.001)**	-0.01 (0.76)	**-0.11 (0.002)**	**-0.07 (0.028)**	**0.08 (0.022)**	**-0.15 (<0.001)**	-0.05 (0.19)	-0.02 (0.64)
Alcohol units/year	**0.08 (0.010)**	0.05 (0.16)	0.06 (0.11)	0.05 (0.16)	-0.05 (0.20)	0.02 (0.58)	0.06 (0.09)	**0.10 (0.006)**
ABI	**0.08 (0.007)**	**0.08 (0.017)**	0.05 (0.14)	0.02 (0.44)	**-0.07 (0.030)**	**0.11 (0.001)**	0.02 (0.56)	-0.01 (0.81)
Carotid IMT	**-0.09 (0.002)**	-0.06 (0.08)	**-0.09 (0.008)**	**-0.10 (0.002)**	0.07 (0.06)	-0.05 (0.13)	-0.01 (0.71)	-0.05 (0.18)
NT-proBNP	**-0.07 (0.012)**	**-0.07 (0.033)**	-0.05 (0.13)	-0.03 (0.38)	0.05 (0.14)	-0.05 (0.11)	-0.05 (0.12)	-0.05 (0.17)
Inflammation factor	**-0.14 (<0.001)**	-0.03 (0.35)	**-0.08 (0.020)**	**-0.10 (0.001)**	**0.12 (<0.001)**	**-0.13 (<0.001)**	**-0.11 (0.001)**	**-0.07 (0.049)**
HbA1c at clinic visit	**-0.08 (0.008)**	0.03 (0.39)	-0.06 (0.09)	-0.05 (0.11)	**0.07 (0.042)**	**-0.07 (0.029)**	-0.05 (0.17)	**-0.12 (<0.001)**
Historical HbA1c	**-0.10 (0.001)**	0.05 (0.15)	**-0.10 (0.004)**	-0.05 (0.10)	**0.11 (0.001)**	**-0.08 (0.011)**	-0.05 (0.11)	**-0.12 (<0.001)**
Cortisol	-0.01 (0.69)	-0.04 (0.22)	-0.03 (0.43)	0.01 (0.78)	0.02 (0.58)	0.02 (0.54)	-0.01 (0.82)	-0.03 (0.44)

Diabetic retinopathy	-0.05 (0.07)	-0.04 (0.17)	-0.03 (0.46)	-0.01 (0.74)	0.05 (0.12)	-0.03 (0.29)	-0.03 (0.38)	-0.04 (0.21)
Severe hypoglycaemia	**-0.08 (0.004)**	0.02 (0.50)	-0.02 (0.53)	**-0.07 (0.018)**	**0.11 (0.001)**	**-0.08 (0.010)**	-0.05 (0.11)	-0.05 (0.12)
Duration of diabetes	**-0.08 (0.002)**	-0.01 (0.69)	**-0.09 (0.008)**	-0.05 (0.08)	**0.07 (0.041)**	**-0.08 (0.018)**	-0.05 (0.12)	**-0.08 (0.010)**
HADS-D	**-0.14 (<0.001)**	-0.05 (0.11)	**-0.03 (0.32)**	**-0.07 (0.023)**	**0.12 (<0.001)**	**-0.14 (<0.001)**	**-0.12 (<0.001)**	**-0.10 (0.002)**

Analyses were linear regression analyses adjusted for age, sex and baseline MHVS. β values are standardised regression correlation coefficients (p-values). β values are standardised regression correlation coefficients (p-values). Values of *g* have been imputed, for individual cognitive tests are non-imputed. Log-transformed variables were used for Trail-Making, HADS-D, NT-proBNP, and duration of diabetes. Square root transformed values were used for packyears. Alcohol units/year is based on quintiles. N = 733 to 814. Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test.

Table D.5: Univariate associations of risk factors with reaction time at year 4 adjusted for MHVS

	Simple Reaction Time mean	Choice Reaction Time mean
Any symptomatic MVD	0.02 (0.66)	0.02 (0.54)
Transient ischaemic attack	0.01 (0.76)	0.01 (0.88)
Stroke	**0.08 (0.020)**	0.03 (0.35)
Myocardial infarction	-0.03 (0.34)	-0.04 (0.33)
Angina	0.00 (0.95)	0.04 (0.30)
Peripheral arterial disease	-0.01 (0.86)	0.07 (0.07)
Waist-hip ratio	**0.11 (0.006)**	**0.15 (<0.001)**
Total cholesterol	-0.03 (0.42)	-0.04 (0.31)
HDL cholesterol	-0.03 (0.36)	-0.04 (0.34)
LDL:HDL	0.01 (0.73)	0.03 (0.38)
Systolic bp at clinic visit	0.05 (0.12)	**0.09 (0.012)**
Diastolic bp at clinic visit	-0.02 (0.55)	-0.02 (0.53)
Historical systolic bp	0.02 (0.55)	0.05 (0.15)
Packyears	0.01 (0.87)	0.05 (0.17)
Alcohol units/year	0.02 (0.67)	0.01 (0.78)
ABI	-0.05 (0.13)	-0.06 (0.13)
Carotid IMT	**0.11 (0.004)**	**0.12 (0.001)**
NT-proBNP	-0.01 (0.72)	0.03 (0.47)
Inflammation factor	0.06 (0.09)	0.06 (0.08)
HbA1c at clinic visit	0.00 (0.95)	0.03 (0.43)
Historical HbA1c	0.06 (0.11)	0.06 (0.11)
Cortisol	-0.05 (0.18)	-0.02 (0.53)
Diabetic retinopathy	-0.01 (0.71)	0.01 (0.71)
Severe hypoglycaemia	**0.09 (0.007)**	**0.08 (0.023)**
Duration of diabetes	0.02 (0.60)	0.03 (0.38)
HADS-D	0.01 (0.77)	**0.07 (0.049)**

Analyses were linear regression analyses adjusted for age, sex and baseline MHVS. β values are standardised regression correlation coefficients (p-values). Log-transformed variables were used for SRT mean, HADS-D, NT-proBNP and duration of diabetes. Square root transformed values were used for packyears. Alcohol units/year is based on quintiles; N = 738 to 788 for SRT and 695 to 740 for CRT.
Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale; SRT, simple reaction time; CRT, choice reaction time.

Risk factors and four-year cognitive change

Table D.6: Univariate associations of risk factors with four-year cognitive change

	'g'	Logical Memory	Faces	Matrix Reasoning	Trail-Making	Digit Symbol Coding	Letter-Number Sequencing	Verbal Fluency
Any symptomatic MVD	**-0.09 (0.008)**	**-0.08 (0.002)**	-0.05 (0.06)	-0.03 (0.29)	**0.06 (0.018)**	**-0.05 (0.046)**	**-0.07 (0.017)**	**-0.05 (0.019)**
Transient ischaemic attack	-0.05 (0.18)	0.01 (0.86)	-0.04 (0.21)	**-0.08 (0.002)**	0.01 (0.68)	-0.01 (0.68)	-0.01 (0.70)	0.00 (0.97)
Stroke	**-0.07 (0.036)**	-0.03 (0.30)	-0.02 (0.59)	-0.03 (0.20)	**0.06 (0.030)**	**-0.06 (0.011)**	-0.04 (0.25)	-0.04 (0.07)
Myocardial infarction	-0.04 (0.29)	-0.03 (0.23)	0.00 (0.88)	-0.04 (0.10)	0.03 (0.19)	-0.02 (0.53)	**-0.07 (0.031)**	-0.01 (0.81)
Angina	-0.04 (0.24)	**-0.06 (0.039)**	-0.02 (0.41)	0.00 (0.93)	0.04 (0.13)	**-0.05 (0.022)**	-0.05 (0.11)	-0.03 (0.15)
Peripheral arterial disease	-0.02 (0.57)	-0.05 (0.08)	-0.05 (0.11)	0.01 (0.70)	0.01 (0.68)	0.02 (0.37)	-0.04 (0.23)	-0.01 (0.70)
Waist-hip ratio	**-0.10 (0.012)**	-0.02 (0.51)	-0.01 (0.81)	-0.05 (0.08)	**0.06 (0.043)**	**-0.10 (<0.001)**	**-0.10 (0.004)**	-0.04 (0.07)
Total cholesterol	0.04 (0.28)	-0.02 (0.49)	0.04 (0.22)	0.00 (0.98)	-0.03 (0.23)	-0.02 (0.51)	0.05 (0.15)	-0.02 (0.45)
HDL cholesterol	0.04 (0.26)	0.04 (0.18)	**0.06 (0.037)**	0.00 (0.96)	-0.04 (0.16)	0.00 (0.95)	**0.07 (0.028)**	-0.01 (0.72)
LDL:HDL	-0.02 (0.49)	-0.05 (0.06)	-0.01 (0.76)	-0.01 (0.65)	0.02 (0.48)	-0.02 (0.34)	-0.02 (0.49)	-0.02 (0.25)
Systolic bp at clinic visit	**-0.10 (0.006)**	-0.04 (0.20)	-0.03 (0.27)	-0.03 (0.23)	0.01 (0.67)	-0.03 (0.23)	**-0.07 (0.014)**	**-0.05 (0.017)**
Diastolic bp at clinic visit	-0.01 (0.86)	0.03 (0.22)	-0.02 (0.59)	0.00 (0.96)	-0.03 (0.19)	0.02 (0.45)	-0.04 (0.16)	-0.01 (0.77)
Historical systolic bp	-0.05 (0.19)	0.00 (0.91)	-0.03 (0.26)	-0.03 (0.19)	0.02 (0.47)	0.02 (0.49)	0.01 (0.80)	-0.02 (0.33)
Packyears	**-0.15 (<0.001)**	-0.05 (0.07)	**-0.09 (0.003)**	-0.06 (0.03)	**0.05 (0.052)**	**-0.08 (0.001)**	**-0.07 (0.036)**	**-0.05 (0.024)**
Alcohol (units/year)	0.02 (0.60)	0.05 (0.11)	0.05 (0.11)	0.04 (0.21)	-0.05 (0.07)	0.01 (0.82)	0.04 (0.25)	0.03 (0.19)
ABI	**0.12 (<0.001)**	**0.08 (0.006)**	0.04 (0.12)	0.02 (0.54)	**-0.06 (0.012)**	**0.07 (0.004)**	0.05 (0.13)	0.03 (0.12)
Carotid IMT	**-0.15 (<0.001)**	**-0.06 (0.030)**	**-0.08 (0.007)**	**-0.08 (0.003)**	0.05 (0.08)	**-0.08 (0.002)**	-0.02 (0.46)	-0.02 (0.50)
NT-proBNP	**-0.12 (0.001)**	**-0.07 (0.013)**	-0.04 (0.15)	-0.01 (0.65)	0.04 (0.11)	**-0.05 (0.053)**	**-0.08 (0.014)**	**-0.07 (0.001)**
Inflammation factor	**-0.17 (<0.001)**	**-0.07 (0.018)**	**-0.06 (0.030)**	**-0.07 (0.006)**	**0.07 (0.012)**	**-0.11 (<0.001)**	**-0.14 (<0.001)**	**-0.06 (0.009)**
HbA1c at clinic visit	**-0.08 (0.025)**	-0.01 (0.81)	-0.05 (0.10)	**-0.06 (0.034)**	0.05 (0.06)	**-0.05 (0.042)**	-0.05 (0.09)	**-0.06 (0.008)**
Historical HbA1c	**-0.11 (0.001)**	-0.01 (0.87)	**-0.60 (0.030)**	**-0.06 (0.034)**	**0.07 (0.006)**	-0.04 (0.12)	**-0.06 (0.036)**	**-0.07 (0.001)**
Cortisol	0.03 (0.44)	**-0.06 (0.025)**	0.01 (0.83)	0.03 (0.23)	0.00 (0.91)	**0.06 (0.013)**	0.01 (0.65)	-0.04 (0.07)

Diabetic retinopathy	-0.03 (0.34)	-0.05 (0.08)	0.00 (0.98)	-0.04 (0.11)	0.04 (0.16)	0.02 (0.46)	-0.02 (0.49)	0.00 (0.94)
Severe hypoglycaemia	**-0.08 (0.020)**	0.00 (0.90)	-0.03 (0.23)	**-0.09 (0.001)**	**0.06 (0.024)**	-0.04 (0.10)	-0.03 (0.37)	-0.02 (0.33)
Duration of diabetes	-0.04 (0.21)	-0.04 (0.19)	-0.03 (0.37)	**-0.06 (0.020)**	0.04 (0.15)	-0.02 (0.53)	-0.04 (0.24)	-0.03 (017)
HADS-D	**-0.08 (0.018)**	-0.04 (0.11)	-0.04 (0.14)	-0.04 (0.14)	**0.05 (0.034)**	**-0.07 (0.005)**	**-0.11 (<0.001)**	**-0.04 (0.033)**

Analyses were linear regression analyses adjusted for age, sex and baseline score. β values are standardised regression correlation coefficients (p-values). Data for *g* have been imputed, for individual cognitive tests are non-imputed.
Log-transformed variables were used for TMT-B, HADS-D, NT-proBNP, alcohol units/year and duration of diabetes. Square root transformed values were used for packyears. Alcohol units/year is based on quintiles. N = 757 to 822. Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; ABI, ankle-brachial pressure index; MVD, macrovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; bp, blood pressure; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale; Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test.

Appendix E: Repetition of selected analyses

Univariate analyses of risk factors and four-year cognitive change using raw change scores

Table E.8: Univariate associations of risk factors with four-year cognitive change scores

	'g'	Logical Memory	Faces	Matrix Reasoning	Trail-Making	Digit Symbol Coding	Letter-Number Sequencing	Verbal Fluency
Any symptomatic MVD	0.02 (0.53)	0.07 (0.054)	0.03 (0.46)	-0.02 (0.54)	-0.06 (0.13)	0.01 (0.79)	0.04 (0.25)	0.06 (0.10)
Transient ischaemic attack	0.04 (0.29)	0.00 (0.94)	0.04 (0.28)	**0.08 (0.017)**	0.00 (0.92)	0.01 (0.81)	0.00 (0.91)	0.01 (0.89)
Stroke	0.01 (0.83)	0.00 (0.97)	-0.10 (0.80)	0.02 (0.65)	-0.04 (0.27)	0.03 (0.34)	0.01 (0.85)	0.04 (0.30)
Myocardial infarction	0.00 (0.99)	0.04 (0.26)	0.03 (0.36)	0.02 (0.63)	-0.01 (0.74)	-0.02 (0.62)	0.06 (0.09)	0.00 (0.96)
Angina	-0.01 (0.89)	0.04 (0.25)	0.01 (0.75)	-0.04 (0.21)	-0.04 (0.26)	0.03 (0.38)	0.02 (0.55)	0.05 (0.20)
Peripheral arterial disease	-0.01 (0.90)	0.06 (0.12)	0.04 (0.24)	-0.02 (0.53)	0.00 (0.96)	-0.05 (0.18)	0.02 (0.57)	0.00 (0.99)
Waist-hip ratio	0.02 (0.66)	0.00 (0.98)	-0.02 (0.56)	0.02 (0.70)	-0.03 (0.51)	0.07 (0.09)	0.07 (0.08)	0.04 (0.33)
Total cholesterol	-0.05 (0.18)	0.01 (0.86)	-0.05 (0.19)	0.02 (0.61)	0.03 (0.39)	0.02 (0.61)	-0.06 (0.09)	0.02 (0.53)
HDL cholesterol	0.00 (0.91)	-0.03 (0.38)	-0.06 (0.10)	0.05 (0.21)	0.04 (0.27)	0.02 (0.61)	-0.07 (0.08)	0.05 (0.21)
LDL:HDL	-0.01 (0.73)	0.04 (0.29)	0.00 (0.99)	-0.02 (0.65)	-0.02 (0.58)	0.01 (0.69)	0.01 (0.88)	0.01 (0.69)
Systolic bp at clinic visit	**0.08 (0.018)**	0.04 (0.23)	0.04 (0.31)	0.03 (0.38)	0.00 (0.90)	0.05 (0.16)	**0.07 (0.048)**	**0.07 (0.042)**
Diastolic bp at clinic visit	0.01 (0.72)	-0.06 (0.11)	0.03 (0.41)	0.01 (0.71)	0.04 (0.23)	-0.02 (0.55)	0.07 (0.07)	0.01 (0.73)
Historical systolic bp	0.07 (0.06)	0.00 (0.92)	0.05 (0.19)	0.06 (0.09)	-0.02 (0.51)	-0.02 (0.61)	0.01 (0.84)	0.04 (0.21)
Packyears	**0.10 (0.009)**	0.05 (0.15)	0.07 (0.07)	0.04 (0.35)	-0.07 (0.07)	**0.08 (0.031)**	0.07 (0.06)	**0.08 (0.036)**
Alcohol units/year	0.07 (0.09)	0.00 (0.94)	-0.02 (0.58)	0.02 (0.66)	0.05 (0.25)	0.04 (0.34)	0.03 (0.49)	0.00 (0.95)
ABI	**-0.09 (0.013)**	-0.04 (0.23)	-0.05 (0.15)	0.02 (0.50)	0.07 (0.06)	-0.05 (0.20)	-0.06 (0.09)	-0.04 (0.23)
Carotid IMT	**0.13 (0.001)**	**0.07 (0.053)**	**0.09 (0.020)**	**0.09 (0.015)**	-0.07 (0.07)	**0.13 (0.001)**	0.04 (0.29)	0.02 (0.68)

NT-proBNP	**0.09 (0.014)**	0.06 (0.08)	0.03 (0.38)	-0.01 (0.80)	-0.04 (0.27)	0.05 (0.17)	**0.08 (0.034)**	**0.12 (0.001)**
Inflammation factor	**0.10 (0.006)**	0.06 (0.09)	0.04 (0.28)	0.04 (0.26)	-0.06 (0.08)	**0.11 (0.002)**	**0.13 (0.001)**	**0.07 (0.042)**
HbA1c at clinic visit	0.03 (0.35)	0.00 (0.99)	0.03 (0.37)	0.05 (0.15)	**-0.08 (0.037)**	0.05 (0.20)	0.03 (0.47)	0.06 (0.09)
Historical HbA1c	**0.07 (0.038)**	0.02 (0.56)	0.04 (0.24)	0.06 (0.10)	**-0.09 (0.011)**	0.03 (0.49)	0.06 (0.09)	**0.09 (0.014)**
Cortisol	-0.03 (0.40)	**0.09 (0.009)**	-0.02 (0.52)	-0.05 (0.20)	-0.01 (0.71)	**-0.10 (0.007)**	-0.03 (0.44)	**0.07 (0.049)**
Diabetic retinopathy	0.00 (0.94)	0.06 (0.11)	-0.01 (0.70)	**0.07 (0.052)**	-0.03 (0.36)	-0.05 (0.16)	0.02 (0.62)	-0.02 (0.61)
Severe hypoglycaemia	0.05 (0.15)	0.01 (0.74)	0.04 (0.21)	**0.11 (0.003)**	**-0.07 (0.037)**	0.03 (0.44)	0.00 (0.96)	0.02 (0.57)
Duration of diabetes	0.00 (0.91)	0.05 (0.14)	-0.01 (0.69)	**0.07 (0.036)**	-0.04 (0.30)	-0.02 (0.68)	0.02 (0.51)	0.02 (0.50)
HADS-D	0.00 (0.93)	**0.09 (0.028)**	0.06 (0.13)	0.06 (0.13)	**-0.09 (0.021)**	**0.13 (0.001)**	**0.13 (0.001)**	**0.12 (0.002)**

β values are standardised regression correlation coefficients (p-values)

Due to a normal distribution of the change variable, untransformed data was used for TMT-B. Data for *g* have been imputed, for individual cognitive tests are non-imputed. Log-transformed variables were used for Trail-Making, NT-proBNP, HADS-D and duration of diabetes. Alcohol units/year is based on quintiles. Packyears is square root transformed. N = 734 to 819.

Analyses are of raw change scores and adjusted for age and sex. Positive values represent decline in scores between baseline and year 4; negative values represent increased scores between baseline and year 4. For the calculation of change in *g*, baseline *g* and follow-up *g* were standardised on the same population (as described for the adjustment method in Section 4.5.3). Prior to calculation of the change score for *g*, follow-up *g* was multiplied by the ratio of variances and the mean difference between baseline and follow-up *g*. This ensured that both baseline and follow-up *g* were measured on the same scale and that latent means and variances were identical. Peripheral arterial disease measured by presence of intermittent claudication; MVD, macrovascular disease; carotid IMT, carotid intima-media thickness; HADS-D, Depression subscale of the Hospital Anxiety and Depression Scale. Verbal Fluency, Borkowski Verbal Fluency Test; Trail-Making, Trail-Making Test-B.

Analyses of incident SH associations with cognitive function and cognitive decline with restriction to first-ever incident SH

For the purpose of excluding subjects who had both a baseline history of SH and incident SH from analyses, patients with incident SH as well as a baseline history of SH were recoded as 'missing' in the analyses reported in this section.

Table E.9: First-ever incident severe hypoglycaemia and cognitive function at year 4

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η^2
Model 1: age, sex				
Matrix Reasoning	**11.95 (11.56 – 12.33)**	**10.16 (8.80 – 11.52)**	**0.014**	**0.008**
Letter-Number	**9.11 (8.89 - 9.33)**	**8.07 (7.28 – 8.85)**	**0.012**	**0.009**
Verbal Fluency	37.54 (36.8 – 38.50)	34.35 (30.98 – 37.73)	0.076	0.004
Digit Symbol Coding	**51.05 (50.02 – 52.08)**	**46.91 (43.23 – 50.59)**	**0.034**	**0.006**
Trail-Making	**107.40 (04.17 – 10.50)**	**26.22 (3.64 – 40.33)**	**0.004**	**0.012**
Faces	**69.59 (68.98 – 70.20)**	**66.78 (64.64 – 68.91)**	**0.013**	**0.009**
Logical Memory	27.40 (26.78 – 8.01)	25.86 (23.68 – 8.04)	0.185	0.002
‘g’	**0.09 (0.01 – 0.16)**	**-0.37 (-0.63 – -0.12)**	**0.001**	**0.016**
Model 2[a]:				
Matrix Reasoning	11.96 (11.57 – 12.36)	10.76 (9.30 – 2.21)	0.120	0.004
Letter-Number	**9.12 (8.89 – 9.34)**	**8.20 (7.38 – 9.02)**	**0.035**	**0.007**
Verbal Fluency	37.72 (36.74 – 38.69)	35.91 (32.33 – 39.49)	0.343	0.001
Digit Symbol Coding	51.05 (50.01 – 52.09)	47.94 (44.08 – 51.80)	0.129	0.004
Trail-Making	**107.02 (103.75 – 110.28)**	**120.78 (107.99 – 134.96)**	**0.042**	**0.006**
Faces	69.67 (69.04 – 70.29)	67.36 (65.06 – 69.66)	0.059	0.005
Logical Memory	27.59 (26.96 – 28.22)	25.38 (23.08 – 27.60)	0.071	0.005
‘g’	**0.10 (0.02 – 0.17)**	**-0.27 (-0.54 – 0.00)**	**0.010**	**0.010**

Values are adjusted means (95% CI). N = 701 to 727 for Model 1; N = 692 to 769 for Model 2. Data on *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making is log-transformed. Means for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Trail-Making, Trail-Making Test-B.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

<u>Table E.10: First-ever incident severe hypoglycaemia and reaction time at year 4</u>

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η^2
Model 1: age, sex				
SRT mean	**312.62 (307.97 – 37.35)**	**335.29 (317.35 – 354.25)**	**0.016**	**0.008**
CRT mean	628.01 (618.92 – 637.10)	650.35 (617.05 – 683.64)	0.205	0.002
Model 2[a]				
SRT mean	**312.31 (307.66 – 317.35)**	**331.96 (313.56 – 351.43)**	**0.046**	**0.006**
CRT mean	627.90 (618.63 – 637.16)	640.95 (606.16 – 675.75)	0.479	0.001

Values are adjusted means (95% CI). N = 704 and 667 for Model 1; N = 671 and 634 for Model 2. Means for ln(SRT) are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time.

[a]adjusted for age, sex, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

Table E.11: First-ever incident severe hypoglycaemia and year 4 cognitive test scores adjusted for baseline MHVS

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η²
Model 1: age, sex, baseline MHVS				
Matrix Reasoning	**11.88 (11.54 – 12.23)**	**10.59 (9.36 – 11.81)**	**0.046**	**0.006**
Letter-Number	**9.10 (8.90 – 9.30)**	**8.34 (7.62 – 9.05)**	**0.045**	**0.006**
Verbal Fluency	37.44 (36.55 – 38.33)	35.43 (32.30 – 38.57)	0.227	0.002
Digit Symbol Coding	50.95 (49.99 – 51.91)	48.11 (44.70 – 51.52)	0.117	0.004
Trail-Making	**107.55 (104.58 – 110.61)**	**122.36 (10.72 – 135.37)**	**0.015**	**0.008**
Faces	**69.57 (68.98 – 70.16)**	**67.07 (65.00 – 69.14)**	**0.023**	**0.007**
Logical Memory	27.34 (26.79 – 27.88)	26.90 (24.95 – 28.85)	0.672	<0.001
'g'	**0.08 (0.02 – 0.14)**	**-0.24 (-0.46 – -0.03)**	**0.004**	**0.012**
Model 2[a]:				
Matrix Reasoning	11.90 (11.55 – 12.25)	11.19 (9.87 – 12.50)	0.307	0.002
Letter-Number	9.11 (8.91 – 9.32)	8.43 (7.67 – 9.19)	0.089	0.004
Verbal Fluency	37.63 (36.73 – 38.53)	37.00 (33.66 – 40.32)	0.722	<0.001
Digit Symbol Coding	50.96 (49.98 – 51.93)	48.98 (45.38 – 52.59)	0.302	0.002
Trail-Making	107.23 (104.17 – 10.39)	117.21 (05.32 – 130.45)	0.116	0.004
Faces	69.65 (69.05 – 70.26)	67.61 (65.37 – 69.86)	0.087	0.004
Logical Memory	27.55 (26.99 – 28.11)	26.24 (24.16 – 28.32)	0.235	0.002
'g'	**0.09 (0.03 – 0.15)**	**-0.16 (0.38 – 0.06)**	**0.035**	**0.007**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 694 to 719 for Model 1; N = 662 to 684 for Model 2. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making is log-transformed. Means for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Digit Symbol, Digit Symbol Coding, Trail-Making, Trail-Making Test-B, MHVS, Mill-Hill Vocabulary Scale.

[a]model 1 +HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy

Table E.12: First-ever incident severe hypoglycaemia and reaction time at year 4 adjusted for MHVS

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial η^2
Model 1: age, sex, baseline MHVS				
SRT mean	**312.94 (308.28 – 317.67)**	**332.95 (35.13 – 351.43)**	**0.032**	**0.007**
CRT mean	628.24 (619.31 – 637.18)	646.82 (614.27 – 679.37)	0.281	0.002
Model 2[a]				
SRT mean	312.94 (308.28 – 317.67)	329.31 (311.38 – 348.63)	0.086	0.005
CRT mean	628.07 (618.95 – 637.19)	638.18 (604.11 – 672.24)	0.576	0.001

Values are adjusted means (95% CI). N = 697 and 660 for Model 1; N = 664 and 627 for Model 2. Means for ln(SRT) are geometric means. SRT and CRT are measured in milliseconds. SRT, simple reaction time; CRT, choice reaction time; MHVS, Mill-Hill Vocabulary Scale.
[a]adjusted for age, sex, MHVS, HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy.

<u>**Table E.13: First-ever incident severe hypoglycaemia and four-year cognitive change**</u>

	No severe hypoglycaemia (n=730)	Severe hypoglycaemia (≥1 episodes; n=85)	p-value for difference	Partial ŋ²
Model 1: age, sex, baseline score				
Matrix Reasoning	11.89 (11.60 – 12.19)	11.01 (9.97 – 12.06)	0.113	0.004
Letter-Number	9.10 (8.91 – 9.28)	8.40 (7.73 – 9.07)	0.051	0.006
Verbal Fluency	**37.57 (37.02 – 38.13)**	**34.29 (32.34 – 36.24)**	**0.002**	**0.014**
Digit Symbol Coding	**50.96 (50.25 – 51.67)**	**48.10 (45.57 – 50.63)**	**0.033**	**0.006**
Trail-Making	**107.55 (105.2 – 109.95)**	**121.27 (120.30 – 13.24)**	**0.004**	**0.011**
Faces	**69.51 (69.01 – 70.00)**	**67.66 (65.90 – 69.41)**	**0.047**	**0.006**
Logical Memory	27.41 (26.94 – 27.89)	26.83 (25.14 – 28.52)	0.515	0.001
'g'	**0.07 (-0.01 – 0.14)**	**-0.35 (-0.62 - -0.09)**	**0.002**	**0.013**
Model 2[a]:				
Matrix Reasoning	11.94 (11.64 – 12.24)	11.30 (10.18 – 12.43)	0.286	0.002
Letter-Number	9.11 (8.91 – 9.30)	8.50 (7.79 – 9.21)	0.109	0.004
Verbal Fluency	**37.84 (37.27 – 38.41)**	**34.74 (32.65 – 36.82)**	**0.005**	**0.012**
Digit Symbol Coding	51.00 (50.27 – 51.72)	48.57 (45.88 – 51.26)	0.089	0.004
Trail-Making	**107.13 (104.69 – 109.62)**	**117.45 (107.99 – 127.87)**	**0.039**	**0.006**
Faces	69.58 (69.08 – 70.09)	68.29 (66.40 – 70.18)	0.196	0.002
Logical Memory	27.58 (27.09 – 28.06)	26.85 (25.05 – 28.64)	0.444	0.001
'g'	**0.06 (-0.01 – 0.14)**	**-0.27 (-0.55 – 0.01)**	**0.023**	**0.008**
Model 3[b]:				
Matrix Reasoning	11.89 (11.60 – 12.19)	11.40 (10.30 – 12.50)	0.396	0.001
Letter-Number	9.11 (8.93 – 9.30)	8.60 (7.91 – 9.28)	0.157	0.003
Verbal Fluency	**37.83 (37.27 – 38.40)**	**34.69 (32.59 – 36.87)**	**0.005**	**0.012**
Digit Symbol Coding	50.97 (50.25 – 51.69)	48.88 (46.21 – 51.54)	0.140	0.003
Trail-Making	107.13 (104.69 – 109.62)	116.40 (106.91 – 126.60)	0.067	0.005
Faces	69.59 (69.08 – 70.09)	68.45 (66.58 – 70.32)	0.253	0.002
Logical Memory	27.58 (27.12 – 28.04)	27.04 (25.32 – 28.75)	0.547	0.001
'g'	**0.07 (-0.01 – 0.14)**	**-0.29 (-0.57 – -0.02)**	**0.014**	**0.009**

Outcome variables are follow-up cognitive test scores. Values are adjusted means (95% CI). N = 694 to 726 for Model 1; N = 663 to 691 for Model 2; N = 658 to 684 for Model 3. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making is log-transformed. Means for this test are geometric means. Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Trail-Making Test-B, MHVS, Mill-Hill Vocabulary Scale.

[a]model 1 + HDL cholesterol, total cholesterol, systolic blood pressure at clinic visit, diastolic blood pressure at clinic visit, smoking (ex/current/never), HbA1c at clinic visit, transient ischaemic attack, stroke, myocardial infarction, angina, retinopathy

[b]model 2 + baseline MHVS

Selected analyses repeated with exclusion of patients with dementia (total n=19; 'attenders' with dementia n=4)

Table D.9: Linear regression analyses of associations between macrovascular disease and MHVS at baseline, including and excluding dementia cases (n=19)

	Stroke* (n= 62)		TIA* (n=31)		MI* (n=150)		Angina* (n=298)		PAD* (n=65)		NT-proBNP		Ankle brachial index		Carotid IMT	
	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia	All sub-jects**	Exclu-ding dementia
MHVS	**-0.06 (0.042)**	**-0.06 (0.048)**	-0.01 (0.85)	-0.02 (0.45)	-0.06 (0.053)	-0.04 (0.18)	**-0.11 (<0.001)**	**-0.11 (<0.001)**	-0.01 (0.78)	-0.01 (0.70)	-0.06 (0.06)	-0.05 (0.10)	**0.09 (0.006)**	**0.09 (0.007)**	0.02 (0.55)	0.02 (0.58)

Outcome measure is baseline MHVS. Analyses adjusted for age, sex. *comparisons are made with subjects free of respective symptomatic MVD category. **as reported in the main text of this thesis (Chapter 6). Values are standardised β coefficients (p-values). MHVS, Mill-Hill Vocabulary Scale; MVD, macrovascular disease; TIA, transient ischaemic attack; MI, myocardial infarction; PAD, peripheral arterial disease; NT-proBNP, N-terminal pro-brain natriuretic peptide; carotid IMT, carotid intima-media thickness. NT-proBNP max. n=1050; ABI max. n=1033; cIMT max. n=917

Table D.10: Linear regression analyses of statistically significant associations of macrovascular disease with four-year cognitive change, including and excluding cases of dementia (n=4)

	Stroke* (n=44)		**NT-proBNP**		**Carotid IMT**		**ABI**	
	All subjects **	**Excluding dementia cases**	**All subjects****	**Excluding dementia cases**	**All subjects ****	**Excluding dementia cases**	**All subjects ****	**Excluding dementia cases**
Logical Memory	-0.03 (0.30)	-0.03 (0.28)	**-0.07 (0.013)**	**-0.07 (0.013)**	**-0.06 (0.030)**	**-0.07 (0.027)**	**0.08 (0.006)**	**0.08 (0.006)**
Faces	-0.02 (0.59)	-0.02 (0.57)	-0.04 (0.15)	-0.04 (0.14)	**-0.08 (0.007)**	**-0.08 (0.006)**	0.04 (0.12)	0.05 (0.11)
Letter-Number	-0.04 (0.25)	-0.04 (0.24)	**-0.08 (0.014)**	**-0.08 (0.010)**	-0.02 (0.46)	-0.02 (0.47)	0.05 (0.13)	0.05 (0.12)
Trail-Making	**0.06 (0.030)**	**0.06 (0.026)**	0.04 (0.11)	0.05 (0.08)	0.05 (0.08)	0.05 (0.08)	**-0.06 (0.012)**	**-0.07 (0.012)**
Verbal Fluency	-0.04 (0.07)	-0.04 (0.07)	**-0.07 (0.001)**	**-0.08 (<0.001)**	-0.02 (0.50)	-0.02 (0.48)	0.03 (0.12)	0.03 (0.12)
Digit Symbol Coding	**-0.06 (0.011)**	**-0.06 (0.010)**	-0.05 (0.053)	**-0.05 (0.049)**	**-0.08 (0.002)**	**-0.08 (0.002)**	**0.07 (0.004)**	**0.07 (0.004)**
Matrix Reasoning	-0.03 (0.20)	-0.03 (0.30)	-0.01 (0.65)	-0.01 (0.65)	**-0.08 (0.003)**	**-0.08 (0.003)**	0.02 (0.54)	0.02 (0.51)
g	**-0.07 (0.036)**	**-0.07 (0.036)**	**-0.12 (0.001)**	**-0.12 (0.001)**	**-0.15 (<0.001)**	**-0.15 (<0.001)**	**0.12 (<0.001)**	**0.12 (<0.001)**

Values are standardised β coefficients (p-values). Outcome variables are year 4 follow-up cognitive test scores. Data for individual cognitive tests are non-imputed; for *g* are imputed. Trail-Making variable is log-transformed. *comparisons are made with subjects free of stroke *[*]as reported in the main text of this thesis (Chapter 6). Analyses adjusted for age, sex, baseline scores. Trail-Making, Trail-Making Test-B; Verbal Fluency, Borkowski Verbal Fluency Test; Letter-Number, Letter Number Sequencing; ABI, ankle brachial index; carotid IMT, carotid intima-media thickness. For stroke, n=784 to 822 in analyses of all subjects and n=780 to 818 in analyses excluding dementia patients. For NT-proBNP, n=773 to 811 in analyses of all subjects and n=769 to 807 in analyses excluding dementia patients. For cIMT, n=734 to 768 for analyses of all subjects and n=730 to 764 for analyses excluding dementia patients. For ABI, n=762 to 797 in analyses of all subjects and n=759 to 794 for analyses excluding dementia patients.

Table D.11: Peak pre-morbid ability estimated by MHVS and baseline global ability as risk factors for severe hypoglycaemia, including and excluding dementia cases (n=19)

	Odds ratio (95% CI, p-value) for baseline history of SH		Odds ratio (95% CI, p-value) for incident SH	
	Including dementia cases	**Excluding dementia cases**	**Including dementia cases**	**Excluding dementia cases**
Lowest tertile baseline MHVS*	1.55** (95% CI 1.04, 2.32, p=0.033)	1.57 (95% CI 1.05, 2.35, p=0.029)	1.13** (95% CI 0.69, 1.83, p=0.63)	1.13 (95% CI 0.70, 1.84, p=0.62)
Lowest tertile baseline *g*	1.89 (95% CI 1.26, 2.85, p=0.002)	1.93 (95% CI 1.28, 2.91, p=0.002)	2.04** (95% CI 1.25, 3.31, p=0.004)	2.04 (95% CI 1.26, 3.32, p=0.004)

*compared with higher two tertiles of distribution. All analyses are age- and sex-adjusted. OR, odds ratio; CI, confidence interval; MHVS, Mill-Hill Vocabulary Scale; SH, severe hypoglycaemia. **as reported in the main text of this thesis (Chapter 7)

Table D.12: Lifetime history of severe hypoglycaemia and four-year cognitive change, including and excluding dementia cases (n=4*)

	Including dementia cases**				Excluding dementia cases (n=4*)			
	No severe hypoglycaemia (n=739)	**Severe hypoglycaemia (≥1 episodes; n=77)**	**p-value**	**Partial η^2**	**No severe hypoglycaemia (n=737)**	**Severe hypoglycaemia (≥1 episodes; n=76)**	**p-value**	**Partial η^2**
MR	**11.78 (11.50 – 12.06)**	**10.19 (9.30 – 11.08)**	**0.001**	**0.014**	**11.81 (11.52 – 12.09)**	**10.21 (9.32 – 1.11)**	**0.001**	**0.014**
Letter-Number	8.99 (8.81 – 9.17)	8.72 (8.13 – 9.30)	0.369	0.001	9.01 (8.83 – 9.19)	8.79 (8.20 – 9.37)	0.479	0.001
Verbal Fluency	37.09 (36.55 – 37.63)	36.20 (34.50 – 37.89)	0.327	0.001	37.13 (36.59 – 37.67)	36.33 (34.62 – 38.04)	0.383	0.001
Digit Symbol	50.44 (49.76 – 51.11)	48.54 (46.41 – 50.70)	0.096	0.004	50.51 (49.83 – 51.19)	48.80 (46.66 – 50.95)	0.137	0.003
Trail-Making	**109.73 (107.45 – 112.17)**	**119.34 (111.39 – 127.74)**	**0.024**	**0.007**	**109.62 (107.23 – 111.94)**	**118.04 (110.28 – 126.47)**	**0.043**	**0.005**
Faces	69.36 (68.88 – 69.84)	69.39 (66.88 – 69.91)	0.232	0.002	69.41 (68.93 – 69.88)	68.44 (66.91 – 69.96)	0.234	0.002
Logical Memory	27.40 (26.94 – 27.86)	27.30 (25.85 – 28.74)	0.896	<0.001	27.42 (26.97 – 27.88)	27.35 (25.89 – 28.80)	0.921	<0.001
'g'	**0.03 (-0.04 – 0.10)**	**-0.25 (-0.48 – -0.02)**	**0.020**	**0.007**	**0.03 (-0.04 – 0.10)**	**-0.23 (-0.46 – -0.01)**	**0.030**	**0.006**

Outcome variables are follow-up cognitive test scores. *data on history of severe hypoglycaemia missing for 3 subjects with dementia. **as reported in the main text of this thesis (Chapter 7). Values are adjusted means (95% CI). N for analyses excluding dementia cases = 767 to 804; N for analyses including dementia cases = 770 to 807. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making is log-transformed. Means for this test are geometric means. MR, Matrix Reasoning; Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Digit Symbol, Digit Symbol Coding, Trail-Making, Trail-Making Test-B.

Table D.13: Incident severe hypoglycaemia and four-year cognitive change, including and excluding dementia cases (n=4*)

	Including dementia cases**				**Excluding dementia cases (n=4*)**			
	No severe hypoglycaemia (n=730)	**Severe hypoglycaemia (≥1 episodes; n=85)**	**p-value**	**Partial η²**	**No severe hypoglycaemia (n=728)**	**Severe hypoglycaemia (≥1 episodes; n=85)**	**p-value**	**Partial η²**
MR	**11.78 (11.49 – 12.06)**	**10.58 (9.72 – 11.44)**	**0.010**	**0.008**	**11.79 (11.51 – 12.08)**	**10.60 (9.73 – 11.45)**	**0.010**	**0.008**
Letter-Number	9.04 (8.86 – 9.22)	8.56 (8.01 – 9.12)	0.102	0.004	9.05 (8.87 – 9.11)	8.57 (8.02 – 9.11)	0.099	0.004
Verbal Fluency	37.20 (36.66 – 37.74)	35.78 (34.17 – 37.93)	0.103	0.003	37.23 (36.67 – 37.78)	35.81 (34.20 – 37.47)	0.101	0.003
Digit Symbol	**50.69 (50.01 – 51.37)**	**48.06 (46.03 – 50.10)**	**0.017**	**0.007**	**50.74 (50.06 – 51.42)**	**48.11 (46.08 – 50.14)**	**0.017**	**0.007**
Trail-Making	**108.96 (106.59 – 111.27)**	**119.10 (111.61 – 127.10)**	**0.011**	**0.008**	**108.85 (106.48 – 111.27)**	**119.10 (111.61 – 127.10)**	**0.011**	**0.008**
Faces	**69.55 (69.08 – 70.03)**	**37.38 (65.94 – 68.82)**	**0.005**	**0.010**	69.59 (69.11 – 70.07)	67.41 (65.97 – 68.85)	**0.005**	**0.010**
Logical Memory	27.45 (26.98 – 27.91)	27.25 (25.85 – 28.65)	0.791	<0.001	27.57 (27.00 – 27.93)	27.26 (25.86 – 28.67)	0.784	<0.001
'g'	**0.04 (-0.03 – 0.12)**	**-0.28 (-0.49 – -0.60)**	**0.006**	**0.009**	**0.04 (-0.03 – 0.12)**	**-0.28 (-0.49 – -0.06)**	**0.006**	**0.009**

Outcome variables are follow-up cognitive test scores. *data on incident severe hypoglycaemia missing for 2 subjects with dementia. **as reported in the main text of this thesis (Chapter 7)

Values are adjusted means (95% CI). N for analyses excluding dementia cases = 766 to 804; N for analyses including dementia cases = 768 to 806. Data for *g* are imputed, for remaining cognitive tests are non-imputed. Trail-Making is log-transformed. Means for this test are geometric means. MR, Matrix Reasoning; Letter-Number, Letter Number Sequencing, Verbal Fluency, Borkowski Verbal Fluency Test, Digit Symbol, Digit Symbol Coding, Trail-Making, Trail-Making Test-B.

Table D.14: Linear regression model of follow-up *g* on risk factors, excluding dementia cases (n=4)

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.27	0.01	<0.001	0.089
Sex*	0.08	0.09	0.093	
+inflammation factor	-0.14	0.04	<0.001	0.036
+alcohol	0.19	0.03	<0.001	0.025
+packyears	-0.15	0.01	<0.001	0.024
+waist-hip ratio	-0.14	0.55	0.001	0.014
+stroke	-0.09	0.16	0.013	0.008
Total R^2				0.197
Age*	-0.27	0.01	<0.001	0.119
Sex*	0.09	0.09	0.040	
HADS-D*	-0.10	0.06	0.005	
+inflammation factor	-0.13	0.04	0.001	0.028
+alcohol	0.18	0.03	<0.001	0.024
+packyears	-0.13	0.01	<0.001	0.019
+waist-hip ratio	-0.12	0.56	0.004	0.011
+stroke	-0.08	0.16	0.027	0.006
Total R^2				0.206

N=669 for both models. *G* has been imputed. [a]statistics are shown for final respective model. [b]R^2 change following addition of predictor into the respective previous model. [*]entered in a first block. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears.

Variables entered in stepwise procedure: PA (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol, baseline history of SH.

CHD, coronary heart disease; PA, peripheral atherosclerosis; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia; HADS-D, Depression Subscale of the Hospital Anxiety and Depression Scale.

Table D.15: Linear regression model of estimated lifetime change in *g*, excluding dementia cases (n=4)

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.30	0.01	<0.001	0.116
Sex*	0.16	0.07	<0.001	
+inflammation factor	-0.11	0.03	<0.001	0.018
+packyears	-0.10	0.03	0.001	0.007
+stroke	-0.07	0.14	0.019	0.006
+alcohol	0.08	0.02	0.011	0.005
+DR severity	-0.08	0.05	0.008	0.004
Total R^2				0.156
Model 2				
Age*	-0.30	0.01	<0.001	0.127
Sex*	0.16	0.07	<0.001	
HADS-D*	-0.06	0.05	0.036	
+inflammation factor	-0.10	0.03	<0.001	0.015
+DR severity	-0.08	0.05	0.010	0.006
+packyears	-0.10	0.01	0.003	0.007
+alcohol	0.08	0.02	0.013	0.006
+stroke	-0.06	0.14	0.034	0.004
Total R^2				0.165

N=662 for both models. In model 1, HbA1c was initially included in the model (R^2change = 0.007) but was 'dropped' as a predictor from the final model. Baseline MHVS was entered in a first block (R^2 change = 0.271; this is not included in total R^2 shown in Table), so that the outcome is follow-up *g* adjusted for baseline MHVS. [a]statistics are shown for final model. [b]R^2 change following addition of predictor into the respective previous model.*entered in a second block. *G* has been imputed. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears. Variables entered in stepwise procedure: PA (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol; baseline history of SH.
CHD, coronary heart disease; PA, peripheral atherosclerosis; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia; HADS-D, Depression Subscale of the Hospital Anxiety and Depression Scale.

Table D.16: Linear regression model of four-year change in *g*, excluding dementia cases (n=4)

	standardised β[a]	Standard Error[a]	p-value[a]	R^2 change[b]
Model 1				
Age*	-0.10	0.01	0.015	0.022
Sex*	0.05	0.08	0.227	
+inflammation	-0.10	0.04	0.015	0.022
+cIMT	-0.12	0.22	0.003	0.016
+packyears	-0.11	0.01	0.006	0.011
+HbA1c	-0.09	0.04	0.013	0.009
+NT-proBNP	-0.08	0.03	0.037	0.006
Total R^2				0.086
Model 2				
Age*	-0.10	0.01	0.016	0.025
Sex*	0.05	0.08	0.244	
HADS-D*	0.02	0.06	0.692	
+inflammation	-0.10	0.04	0.014	0.020
+cIMT	-0.12	0.22	0.003	0.016
+packyears	-0.11	0.01	0.006	0.011
+HbA1c	-0.10	0.04	0.012	0.009
+NT-proBNP	-0.09	0.03	0.034	0.006
Total R^2				0.086
Model 3				
Age*	-0.13	0.01	0.001	0.051
Sex*	0.06	0.08	0.17	
MHVS*	0.15	0.01	<0.001	
+cIMT	-0.13	0.22	0.001	0.019
+inflammation	-0.10	0.04	0.009	0.015
+HbA1c	-0.10	0.04	0.011	0.009
+packyears	-0.09	0.01	0.019	0.008
+NT-proBNP	--	--	--	--
Total R^2				0.102

N=668 for Model 1 and Model 2. N=662 for Model 3. Outcome variable is follow-up *g* adjusted for baseline *g*. *G* has been imputed. [a]statistics are shown for final model. [b]R^2 change following addition of predictor into the respective previous model. *entered in a first block. Log-transformed values were used for NT-proBNP; square root transformed values were used for packyears. Variables entered in stepwise procedure: PAD (presence of claudication and/or ABI≤0.90), CHD (angina and/or MI), stroke, cIMT, NT-proBNP, DR, packyears, alcohol consumption (quintiles), waist-hip ratio, LDL:HDL, systolic blood pressure, HbA1c, inflammation factor, cortisol, baseline history of SH. CHD, coronary heart disease; PAD, peripheral arterial disease; LDL:HDL, low-density lipoprotein to high-density lipoprotein ratio; ABI, ankle brachial pressure index; DR, diabetic retinopathy; SH, severe hypoglycaemia.

Appendix F: Unadjusted mean cognitive test performance at year 4 according to hypoglycaemia groups

Table F.1: Unadjusted follow-up cognitive test performance at year 4 according to severe hypoglycaemia

	Baseline history of SH		Incident SH	
	No severe hypoglycaemia	**Severe hypoglycaemia (≥1 episodes)**	**No severe hypoglycaemia**	**Severe hypoglycaemia (≥1 episodes)**
Logical Memory	27.26 ± 8.20	27.95 ± 8.27	27.36 ± 2.26	27.37 ± 7.72
Faces	69.31 ± 8.33	69.08 ± 9.02	69.51 ± 8.27	67.94 ± 9.17
Letter-Number	9.00 ± 2.09	8.45 ± 2.72	9.05 ± 2.89	8.30 ± 2.53
Trail-Making (s)	105.49 (81.78 – 139.50)	112.41 (94.62 – 173.33)	105.01 (81.97 – 138.43)	117.51 (91.34 – 152.04)
Verbal Fluency	37.21 ± 12.76	34.73 ± 12.75	37.19 ±12.60	35.63 ± 13.38
Digit Symbol Coding	50.59 ± 14.06	47.00 ± 14.34	50.65 ± 13.96	48.39 ± 14.09
Matrix Reasoning	11.77 ± 5.25	10.07 ± 4.77	11.81 ± 5.25	10.08 ± 4.63
g	0.04 ± 0.99	-0.23 ± 1.03	0.05 ±0.98	-0.23 ± 1.02

Values are mean ± SD or median (interquartile range). SH, severe hypoglycaemia; Trail-Making, Trail-Making Test-B; Letter-Number, Letter-Number Sequencing.

Table F.2: Unadjusted reaction time test performance at year 4 according to severe hypoglycaemia

	Baseline history of SH		Incident SH	
	No severe hypoglycaemia (n=739)	**Severe hypoglycaemia (≥1 episodes; n=77)**	**No severe hypoglycaemia (n=730)**	**Severe hypoglycaemia (≥1 episodes; n=85)**
SRT mean	303.95 (274.90 – 342.33)	325.40 (280.95 – 374.35)	304.10 (275.85 – 342.25)	328.43 (282.30 – 372.31)
CRT mean	629.41 ± 117.83	665.81 ± 164.00	630.69 ± 117.98	656.02 ± 153.63

Values are mean ± SD or median (interquartile range). SH, severe hypoglycaemia; SRT, simple reaction time; CRT, choice reaction time.

Appendix G: Material of cognitive test battery and Hospital Anxiety and Depression Scale (HADS) administered at baseline and at year 4

Mill-Hill Vocabulary Test

TOMATO fly crack wood dunce <u>fruit</u> step	11 **CONNECT** accident join lace bean flint field	21 **DWINDLE** swindle pander diminish wheeze linger compare
2 **REST** lie down sing go away taste run up cry	12 **PROVIDE** harmonize commit hurt supply annoy divide	22 **LAVISH** unaccountable selfish romantic lawful extravagant praise
3 **PATCH** switch watch hand bang mend cook	13 **STUBBORN** obstinate steady hopeful hollow orderly slack	23 **WHIM** complain noise tonic fancy wind rush
4 **AFRAID** pleased warm goodness horse tired frightened	14 **SCHOONER** building man ship singer plant scholar	24 **SURMOUNT** mountain descend overcome concede appease snub
5 **CRUEL** clean green pretty found water unkind	15 **LIBERTY** worry freedom rich serviette forest cheerful	25 **BOMBASTIC** democratic pompous bickering cautious destructive anxious
6 **BLAZE** kitchen flare grass roof coat side	16 **COURTEOUS** dreadful proud truthful short curtsey polite	26 **RECUMBENT** fugitive cumbersome unwieldy repelling reclining penitent
7 **ACHE** screen tree prize pain boom land	17 **RESEMBLANCE** attendance fondness assemble repose likeness memory	27 **ENVISAGE** contemplate activate surround estrange enfeeble regress
8 **SQUABBLE** quarrel lift bubble photo mould saw	18 **THRIVE** flourish try thrash reap think blame	28 **TRUMPERY** worthless heraldry etiquette highest amusement final
9 **RAGE** crease love invite anger rain hoist	19 **PRECISE** natural stupid faulty grand small exact	29 **GLOWER** extinguish shine disguise gloat aerate scowl
10 **SHRIVEL** linger heed volunteer haunt wither shiver	20 **ELEVATE** revolve move raise work waver disperse	30 **PERPETRATE** appropriate commit propitiate deface control pierce

31 **LEVITY** parsimony velleity salutary frivolity alacrity tariff	35 **TEMERITY** impermanence rashness nervousness stability punctuality submissiveness	39 **VAGARY** vagabond caprice obscurity vulgarity evasion fallacy
32 **LIBERTINE** missionary rescuer profligate canard regicide farrago	36 **FECUND** esculent optative profound prolific sublime salic	40 **SPECIOUS** fallacious coeval palatial typical nutritious flexible
33 **AMULET** savoury jacket flirtation crest cameo charm	37 **ABNEGATE** contradict decry renounce execute believe assemble	41 **SEDULOUS** rebellious dilatory complaisant diligent seductive credulous
34 **QUERULOUS** astringent fearful petulant curious inquiring spurious	38 **TRADUCE** challenge attenuate suspend establish misrepresent conclude	42 **NUGATORY** inimitable adamant sublime contrary numismatic trifling
		43 **ADUMBRATE** foreshadow protect detect eradicate elaborate approach
		44 **MINATORY** implacable diminutive belittling quiescent depository threatening

Mini-Mental State Examination

<u>Orientation-Time</u>
Score one point for each correct answer

		Correct?	
1.	What day of the week is it?	Yes	No
	What date is it today?		
Day		Yes	No
Month		Yes	No
Year		Yes	No
5.	What is the season? *(Allow flexibility when season changes)*	Yes	No

Total correct………….

<u>Orientation-Place</u>
Score one point for each correct answer

		Correct?	
6.	Can you tell me where we are now? For instance, what county or region we are in?	Yes	No
7.	What is the name of this town (city)?	Yes	No
8.	Which country are we in?	Yes	No
9.	What floor of the building are we on?	Yes	No
	What is the name of this place? (or: What is this address? If the subject is tested at home)	Yes	No

Total correct………….

<u>Memory-registration</u>

I am going to name three objects. After I have finished saying all three, I want you to repeat them. Remember what they are because I am going to ask you to name them again in a few minutes.

Name the following three objects taking one second to say each:
LEMON, KEY, BALL. Go!

Note items which are correct on the FIRST attempt and enter number correct under total.

	Correct?	
Lemon	Yes	No
Key	Yes	No
Ball	Yes	No

Total correct………….

Attention and Calculation

Spell ‘world’ backwards Correct?

Yes No

Total correct………….

Memory-Recall

Could you please tell me the three objects
I named earlier Correct?

	Correct?	
Lemon	Yes	No
Key	Yes	No
Ball	Yes	No

Total correct…………

Could you please tell me what this is Correct?

show WATCH	Yes	No
show PENCIL	Yes	No

Total correct…………

I would like you to repeat the following
‘No ifs, ands or buts’ Correct?

Yes No

Total correct…………

Please read and obey the following:
Give the patient the paper with the printed sentence ‘Close your eyes’
Ask the patient to read it and do what it says (only score when patient actually closes eyes)

Correct?

Yes No

Total correct………..

Praxis-ideational

Read the following statement and then hand to the subject a sheet of paper. Make a point of handing to the subject’s midline.

Please listen carefully to the instructions as I will explain it only once:
I am going to give you a piece of paper. When I do, take the paper in your RIGHT hand. Fold the paper in half with both hands, and put the paper down in your lap.

Do not repeat instructions or coach. Score a move as correct only if it takes place n the correct sequence. Note each correct move and enter total number correct (Maximum score=3 points).

	Correct?	
Right hand	Yes	No
Folds	Yes	No
On lap	Yes	No

Total correct……………

Praxis-copying and drawing

Correct?

19. Copy this design (pattern) Yes No

Total correct……………

Praxis-Writing: Spontaneous

Correct?

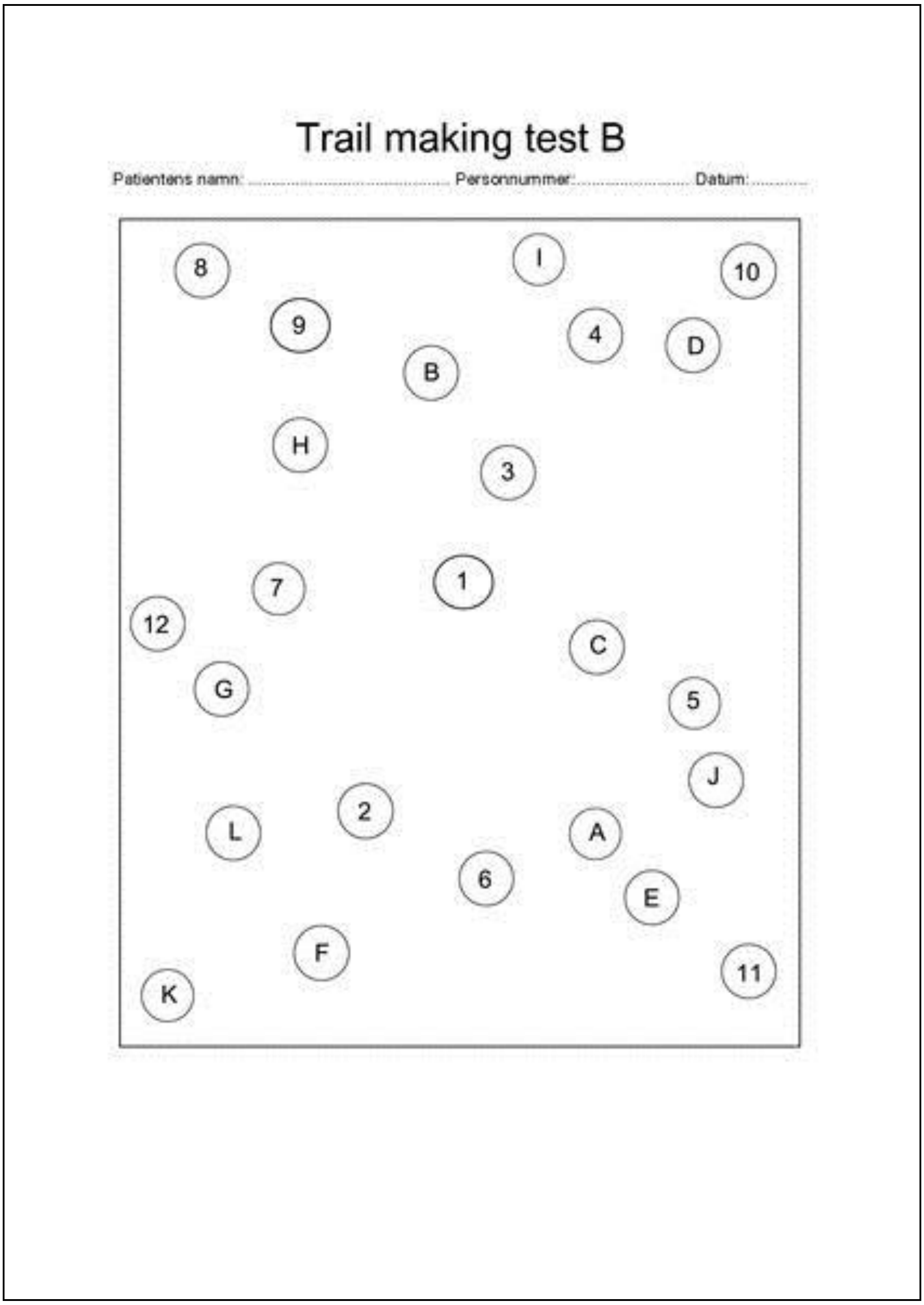
Write a complete sentence in the space

on the page indicated. It can be anything you like as long as it is a complete sentence.	Yes	No

Total correct……………

Overall total correct……………

Trail-Making Test-B



Hospital Anxiety and Depression Scale (HADS)

Tick the response which comes closest to how you have felt in the last few days.

I feel tense or 'wound up'.
- Most of the time………………….
- A lot of the time………………….
- From time to time, occasionally….
- Not at all………………………….

I still enjoy the things I used to enjoy.
- Definitely as much………………
- Not quite so much……………….
- Only a little………………………
- Hardly at all……………………...

I get a sort of frightened feeling as if something awful is about to happen.
- Definitely……………………………..
- Very definitely and quite badly….
- Yes, but not too badly……………
- A little, but it doesn't worry me….
- Not at all………………………….

I can laugh and see the funny side of things.
- As much as I always could………
- Not quite so much now…………..
- Definitely not so much now……...
- Not at all………………………….

Worrying thoughts go through my mind.
- A great deal of the time…………
- A lot of the time…………………
- From time to time but not too often….
- Only occasionally………………..

I feel cheerful.
- Not at all………………………..
- Not often………………………..
- Sometimes………………………

I feel as if I am slowed down.
- Nearly all the time…………..
- Very often…………………...
- Sometimes…………………...
- Not at all……………………

I get a sort of frightened feeling like 'butterflies' in the stomach.
- Not at all……………………
- Occasionally………………..
- Quite often………………….
- Very often…………………..

I have lost interest in my appearance.
- I don't take so much care as I should...
- I may not take quite as much care……
- I take just as much care as ever………

I feel restless as if I have to be on the move.
- Very much indeed…………….
- Quite a lot…………………….
- Not very much………………..
- Not at all………………………

I look forward with enjoyment to things.
- As much as I ever did…………
- Rather less than I used to………
- Definitely less than I used to…..
- Hardly at all…………………….

I get sudden feelings of panic.
- Very often indeed………………
- Quite often……………………..
- Not very often………………….

Most of the time………………...

I can sit at ease and feel relaxed.

Definitely………………………

Usually…………………………

Not often……………………….

Not at all………………………..

Not at all……………………….

I can enjoy a good book or radio or TV programme.

Often…………………………..

Sometimes……………………..

Not often………………………

Very seldom…………………...

Appendix H: Questionnaires administered at baseline

EDINBURGH TYPE 2 DIABETES STUDY (Baseline)

Data Collection Form & Checklist

Subject Study No. ……………………………………….

Date………………………………

PART 1 – Consent and venepuncture

Recorder: DJ GB SL KB CM RM Other……. *(circle initials)*

☐ General consent form signed?

☐ Genetic consent form signed?

☐ Urine sample received from subject and **labelled**?

☐ Questionnaire part 1 received from subject, **checked** and **labelled**?

☐ Patient fasted and still to take diabetic medication (if appropriate)?

If any of the above NOT as per protocol, specify (giving reason and action taken)

……………………………………………………………………………………………….

………………………………………………………………………………………………

Venepuncture

		Yes	No
1.	Was venepuncture completed and all tubes filled?	☐	☐
2.	Was venepuncture difficult/slow?	☐	☐

If any problems and/or tubes not filled, specify ……………………………………….

……………………………………………………………………………………………….

☐ **All** samples labelled?

☐ Correct brown, yellow and red tubes placed in specimen bag with **COMPLETED** request form and urine specimen?

☐ Remaining samples taken to sample processing room and placed on ice?

Subject breakfast

☐ Subject reminded to take medication if necessary?

☐ Questionnaire part 3 (and pen) given to subject?

PART 2 – Cognitive function testing

Tester: DJ GB SL KB CM RM Other…….

Preliminary tests:

Distance vision

	YES	NO
Does subject normally wear glasses/contact lenses for distance vision?	☐	☐
If YES, subject tested wearing glasses/contact lenses?	☐	☐

Right eye ……………….

Left eye ……………….

Near vision

	YES	NO
Does subject normally wear glasses for near vision?	☐	☐
If YES, subject tested wearing glasses?	☐	☐

Right eye N ….

Left eye N ….

BM ☐☐.☐ *(If < 4.0, re-arrange appointment)*

Cognitive Test Battery:

Time tests started………………am

Tick box if complete   If test incomplete/difficulty encountered, specify

☐ Hospital Anxiety & Depression Scale
…………………………………………………………..

☐ Mini-Mental State Examination
……………………………………………………….

☐ Logical memory 1 (WMS-III)
……………………………………………………….

☐ Trail-Making Test B (TMB)
……………………………………………………….

☐ Faces 1 (WMS-III)
……………………………………………………….

☐ Matrix Reasoning (WAIS-III)
……………………………………………………….

☐ Digit symbol test (WAIS-III)
……………………………………………………….

☐ Borkowski Verbal Fluency Task
……………………………………………………….

☐ Mill Hill Vocabulary Scale
……………………………………………………….

☐ Letter-number sequencing (WAIS-III)
……………………………………………………….

☐ Logical memory 2 (WMS-III)
……………………………………………………….

☐ Faces 2 (WMS-III)
……………………………………………………….

PART 3 –physiological testing

Time physiological testing started ………………………………

Tester: DJ GB SL KB CM Other…….

Group A tests (subject lying down)

<u>ABPI</u>
BP measurements **using doppler**

	Left	Right	
Posterior tibial			LOWEST
Dorsalis pedis			
Brachial			HIGHER

Circle lowest ankle and higher arm reading

ABPI = .

RESTING BLOOD PRESSURE

Right arm, **using stethoscope**

	Observed	
Systolic mmHg	☐☐☐	mm/Hg
Diastolic mmHg	☐☐☐	mm Hg

ECG

	Yes	No
ECG recorded and **labelled**	☐	☐

NEUROTHESIOMETRY

	Right big toe		Left big toe	
Reading 1	☐☐.☐	volts	☐☐.☐	volts
Reading 2	☐☐.☐	volts	☐☐.☐	volts
Reading 3	☐☐.☐	volts	☐☐.☐	volts
Average	☐☐.☐	volts	☐☐.☐	volts

Is there any obvious FOOT ulceration?

	Yes	No
Right foot	☐	☐
Left foot	☐	☐

Has subject **ever** had a FOOT ulcer?

	Yes	No
Right foot	☐	☐
Left foot	☐	☐

Group B tests (subject standing)

WAIST CIRCUMFERENCE

Circumference mid-way between lower rib and iliac crest *(feet 30cm apart)*

Reading 1 ☐☐☐ . ☐ cm *(to nearest 0.5cm)*

Reading 2 ☐☐☐ . ☐ cm *(to nearest 0.5cm)*

Average ☐☐☐ . ☐ cm

HIP CIRCUMFERENCE

Max circumference at hips *(feet together)*

Reading 1 ☐☐☐ . ☐ cm *(to nearest 0.5cm)*

Reading 2 ☐☐☐ . ☐ cm *(to nearest 0.5cm)*

Average ☐☐☐ . ☐ cm

HEIGHT

Height (without shoes) ☐☐☐ . ☐ cm

WEIGHT

Weight (without coat and shoes) ☐☐☐ . ☐ kg

BIO-IMPEDENCE

(Shoes off, feet 30 cm apart)

% fat

Reading 1 ☐☐ . ☐ %

Reading 2 ☐☐ . ☐ %

Reading 3 ☐☐ . ☐ %

Average ☐☐ . ☐ %

PART 4 – questionnaire checks and departure

Recorder: DJ GB SL KB CM Other…….

☐ Questionnaire part 2 completed with subject?

☐ Questionnaire part 3 completed and **checked**

OR

☐ sent home with subject?

☐ Appointment for eye pavilion made?

☐ Travel expenses offered?

EDINBURGH TYPE 2 DIABETES STUDY

BASELINE

QUESTIONNAIRE

PLEASE NOTE: ONE OF OUR RESEARCH NURSES WILL GO OVER THE QUESTIONNAIRE WITH YOU AT THE CLINIC AND MAY ASK A FEW ADDITIIONAL QUESTIONS

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this questionnaire will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: ………………………………………………………………..

FORENAMES: ………………………………………………………………..

DATE: ………………………………………………………………..

If you have any difficulties in answering some of the questions, you will have a chance to discuss these with a member of the study team.

THANK YOU FOR YOUR CO-OPERATION IN THIS STUDY

BASELINE QUESTIONNAIRE

IT IS IMPORTANT TO ANSWER ALL THE QUESTIONS CAREFULLY. PLEASE TAKE YOUR TIME.

PERSONAL HISTORY

1. Please tick one box: Male ☐ Female ☐

2. Enter your date of birth: Day ☐☐ Month ☐☐ Year ☐☐

3. Please tick the box showing your present marital status:

Married and/or living with long-term partner ☐
Single ☐
Widowed ☐
Divorced or separated ☐

4. Please enter your address (including postcode) and telephone no.

Address: ……………………………………………………………………

……………………………………………………………………

Postcode: ………………………………..

Telephone no: …………………………………

5. Please enter the details of your GP

GP name: ……………………………………………………………………

Address: ……………………………………………………………………

……………………………………………………………………

EDUCATION

6. What is the HIGHEST level of education you and your spouse/ex-spouse or long-term partner have completed?

Please tick appropriate boxes:

	You	Spouse/ ex-spouse
University/college degree course	☐	☐
Other professional/technical qualification after leaving school	☐	☐
Secondary school	☐	☐
Primary school	☐	☐

ETHNICITY

7. What is your ethnic group?

Please choose ONE section from 1 to 5, then tick the appropriate box to indicate your ethnic Group

(i) White

☐ British
☐ Any Other White background, *please write in*

____

(ii) Mixed

☐ White and Black Caribbean
☐ White and Black African
☐ White and Asian
☐ Any Other Mixed background, *please write in*

____

(iii) Asian or Asian British

☐ Indian
☐ Pakistani
☐ Bangladeshi
☐ Any Other Asian background, *please write in*

____

(iv) Black or Black British

☐ Caribbean
☐ African
☐ Any Other Black background, *please write in*

____

(v) Chinese or other ethnic group

☐ Chinese
☐ Any Other, *please write in* ______________________________

CURRENT EMPOYMENT STATUS

8. At the moment, what is the employment status of you and your spouse/ex-spouse or long-term partner?

You **Spouse/ex-spouse/partner**

☐ Employed, full-time	☐ Employed, full-time
☐ Employed, part-time	☐ Employed, part-time
☐ Unemployed	☐ Unemployed
☐ Retired	☐ Retired
☐ A Housewife (full-time)	☐ A Housewife (full-time)
☐ Other	☐ Other
please specify ……………………………..	please specify ...………………………………..…

MEDICAL HISTORY

Diabetes history

9. When was your diabetes diagnosed (if known)? Year ……..……

10. What treatment do you receive currently for your diabetes?

Yes No

(i) Tablets ☐ ☐

If 'yes', please give name(s) …………………...

Yes No

(ii) Insulin injections ☐ ☐

If 'yes',

(a) give total number of units per day …………………..…units/day

(b) give date (year) when you started insulin year …………………

Yes No Don't Know

11. Have you **ever** had an episode of low blood glucose (hypoglycaemia) ☐ ☐ ☐

when you have needed **someone else** to treat you eg. give sugary drink or glucagon?

If 'yes', how many times has this ever happened?

1-2 ☐

3-4 ☐

5 or over ☐

How many times has this happened **over the past year**?

1-2 ☐

3-4 ☐

5 or over ☐

12. Are you on any regular medical treatment from a doctor as follows:

	Yes	No	Don't Know
Aspirin?	☐	☐	☐
Drugs for angina (including spray)?	☐	☐	☐
Drugs to lower blood pressure?	☐	☐	☐
Drugs to lower cholesterol?	☐	☐	☐

(If you have answered YES to any of these, please include details below)

13. Give names of <u>all</u> current medication if possible (including regular skin creams, eye drops, inhalers, tablets and injections which may or may not be repeat prescriptions):

…………………………………………… ………………………………………………....

…………………………………………… ………………………………………………....

…………………………………………… ………………………………………………....

…………………………………………… ………………………………………………....

	Yes	No	Don't Know
14. Have you taken any oral steroids, used steroid inhalers or used steroid containing creams or eye drops in the last 3 months?	☐	☐	☐

Vascular Disease

15. Have you ever been <u>told by a doctor</u> that you have or have had any of the following?

	Yes	No	Don't Know
(i) Heart attack (coronary thrombosis, myocardial infarction)?	☐	☐	☐
(ii) Angina?	☐	☐	☐
(iii) Stroke?	☐	☐	☐
(iv) Hardening of the arteries in the legs?	☐	☐	☐
(v) High blood pressure?	☐	☐	☐

If you have answered 'yes' to any of the above, please give the year in which the event occurred and/or condition was diagnosed (as near as you can remember) and the name of the hospital/GP surgery where you were/are treated for the condition

Event/condition	Year of event/diagnosis	Hospital/GP surgery where treated
……………………………	……………………….	……………………………
……………………………	………………………..	……………………………

16. Have you ever undergone any of the following procedures/operations?

Don't

Yes No

Know

(i) An operation or balloon treatment to relieve a blockage in ☐ ☐ ☐

the arteries of your heart (coronary by-pass or angioplasty)?

(ii) An operation or balloon treatment to relieve a blockage in ☐ ☐ ☐

the arteries of your leg(s) , other than for varicose veins?

(iii) Surgery to remove toes or leg (above or below the knee)? ☐ ☐ ☐

(iv) An operation or balloon treatment to relieve a blockage in the arteries of your neck (carotid surgery/angioplasty/stenting)? ☐ ☐ ☐

If you have answered 'yes' to any of the above, please give the year in which the procedure was performed and the name of the hospital you attended

Procedure/operation	Year performed	Hospital attended
……………………………	……………………….	…………………………
……………………………	………………………..	…………………………

Liver Condition/Disease

17. Have you ever been <u>told by a doctor</u> that you have or have had any of the following?

Yes No Don't Know

(i) Hepatitis? ☐ ☐ ☐

(ii) Cirrhosis of the liver? ☐ ☐ ☐

(iii) Any other disease/medical condition affecting the liver? ☐ ☐ ☐

If you have answered 'yes' to any of the above, please give the name of the condition, the year in which it was diagnosed (as near as you can remember) and the name of the hospital where you were/are treated for the condition

Name of condition	Year of diagnosis	Hospital where treated
……………………………	……………………….	…………………………
……………………………	………………………..	…………………………

18. Have you ever had any of the following investigations of your liver

Yes No Don't Know

(i) Abnormal blood tests of liver function? ☐ ☐ ☐

(ii) Liver biopsy? ☐ ☐ ☐

(iii) Scan (ultrasound or CT etc.) of the liver? ☐ ☐ ☐

(iv) Other investigation of the liver? ☐ ☐ ☐

If you have answered 'yes' to any of the above, please give the name of the investigation, the year in which it was done (as near as you can remember) and the name of the hospital where the test/investigation was performed

Name of investigation	Year done	Hospital where performed
……………………………	……………………….	…………………………
……………………………	………………………..	…………………………

Other Medical Conditions

Yes No Don't Know

19. Do you suffer from disease of the thyroid gland? ☐ ☐ ☐

20. Do you have any other medical conditions not mentioned above? ☐ ☐

If yes, please specify: …………………………………………………………………..

……………………………………………………………………………………………………

……………………………………………………………………………………………..

ALCOHOL

21. **Current alcohol intake**

(i) Think back carefully over the last seven days. Please write in each column the exact number of alcoholic drinks you consumed on each day during the past week. If none consumed write '0' in the boxes.

Try to remember where and who you were with on each day. This may help you remember what you had to drink.

	Pints of beer, lager, cider etc	Single glasses of whisky, vodka, gin etc	Single glasses of martini, wine, sherry, etc
Monday			
Tuesday			
Wednesday			
Thursday			
Friday			
Saturday			
Sunday			

(ii) Would you say that last week was fairly typical of what you usually have to drink in a week? Yes ☐ No ☐

(iii) If last week was not typical, would you normally drink more or less in a week? More ☐ Less ☐

22. **Alcohol intake over past year**

(i) How often did you have a drink containing alcohol in the past year?
Consider a "drink" to be a can or bottle of beer, a glass of wine, or one cocktail or a measure of spirits (like scotch, gin, or vodka).

never ☐
monthly or less ☐
2 to 4 times a month ☐
2 to 3 times a week ☐
4 to 5 times a week ☐
6 or more times a week ☐

(ii) How many drinks did you have on a typical day when you were drinking in the past year?

0 drinks ☐
1 to 2 drinks ☐
3 to 4 drinks ☐
5 to 6 drinks ☐
7 to 9 drinks ☐
10 or more drinks ☐

(iii) How often did you have 6 or more drinks on one occasion in the past year?

never ☐
less than monthly ☐
monthly ☐
weekly ☐
daily or almost daily ☐

23. Have you or your doctor ever considered that you suffer/have suffered in the past from an alcohol problem/excessive drinking? Yes ☐ No ☐

SMOKING

Smoking has been linked with many health problems. It is important that you answer the following section as accurately as possible.

24. Do you smoke at present? Yes ☐ No ☐

If no, proceed to Question 29

25. What do you usually smoke now?

	Yes	No
Cigarettes	☐	☐
Pipe	☐	☐
Cigars	☐	☐

26. How many do you usually smoke now?

Cigarettes per day ……… cigarettes

Ozs. tobacco per week ……… ozs.

Cigars per week ……… cigars

27. For how many years during your life have you smoked cigarettes? ……… years

28. How many cigarettes have you smoked on average per day during the period you have smoked? ………cigarettes

Now proceed to Question 34

Yes No

29. Have you ever smoked regularly? ☐ ☐

If no, proceed to Question 34

30. What did you usually smoke? Yes No

	Yes	No
Cigarettes	☐	☐
Pipe	☐	☐
Cigars	☐	☐

31. How much did you smoke on average while you were a smoker?

Cigarettes per day ……… cigarettes

Ozs. tobacco per week ……… oz.

Cigars per week ……… cigars

32. For how many years did you smoke cigarettes? ……… years

33. If you smoked cigarettes, how long is it since you finally gave up?

………. years ……… months

CHEST PAIN

Yes No

34. Do you ever get pain or discomfort in your chest? ☐ ☐

IF NO, PROCEED TO QUESTION 40

Yes No

35. Do you get this pain or discomfort when you walk uphill or hurry? ☐ ☐

IF NO, PROCEED TO QUESTION 40

Yes No

36. Do you get it when you walk at an ordinary pace on the level? ☐ ☐

37. When you get any pain or discomfort in your chest what do you do?

Tick one

Stop ☐

Slow down ☐

Continue at the same pace ☐

Yes No

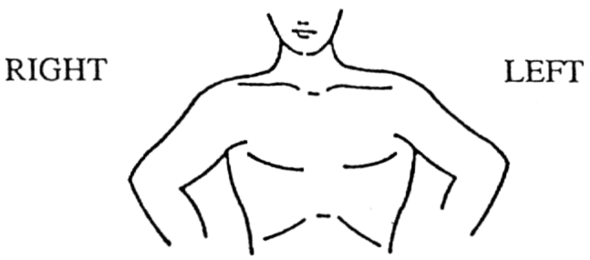
38. Does it go away when you stand still or sit down? ☐ ☐

How soon? Tick one

10 minutes or less ☐

More than 10 minutes ☐

39. Where do you get this pain or discomfort? Mark the place(s) with an 'X' on the diagram



Yes No

40. (i) Have you ever had a severe pain across the front of your chest lasting for half an hour? ☐ ☐

(ii) What was the cause? ………………………………………………………………

LEG PAIN

Yes No I am unable to walk

41. Do you get a pain or discomfort in your leg(s) when you walk? ☐ ☐

If you answered 'yes' to question 41, please answer the following questions.

Yes No

(i) Does this pain ever begin when you are standing still or sitting? ☐ ☐

(ii) Do you get it if you walk uphill or hurry? ☐ ☐

(iii) Do you get it when you walk at an ordinary pace on the level? ☐ ☐

(iv) Does the pain ever disappear while you are still walking? ☐ ☐

(v) What do you do if you get it when you are walking? ☐

Tick one

Stop ☐

Slow down ☐

Continue at same pace ☐

(vi) What happens to it if you stand still?

Tick one

Usually continues for more than 10 minutes ☐

Usually disappears in 10 minutes or less ☐

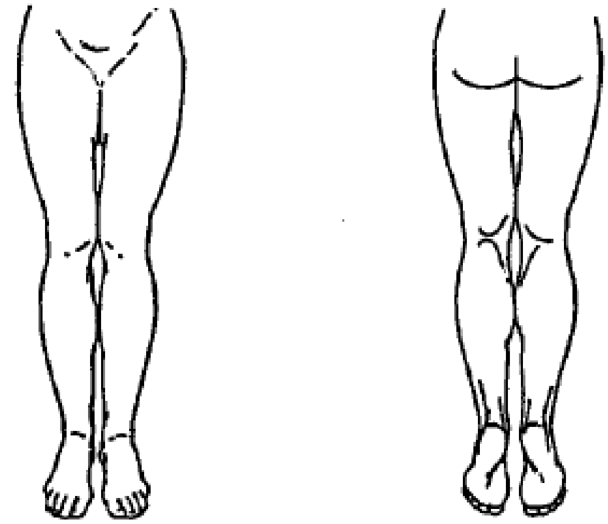
(vii) Where do you get this pain or discomfort?

Yes No

(i) Do you get this pain in your calf (or calves)? ☐ ☐

(ii) Please mark the place(s) where you get the pain with 'X' on the diagram below

Front



Back

EDINBURGH TYPE 2 DIABETES STUDY

BASELINE QUESTIONNAIRE

Part 2

THE INFORMATION IN THIS QUESTIONNAIRE IS HIGHLY CONFIDENTIAL AND IS PART OF A MEDICAL RESEARCH STUDY

The information you give in this questionnaire will be treated as strictly confidential and will be available only to your own doctor and the study team. The results of the research will appear only in the form of general statistics from which it will be impossible to identify you as an individual.

Please complete the following:

SURNAME: …………………………………………………………………..

FORENAMES: …………………………………………………………………..

DATE: …………………………………………………………………..

EMPLOYMENT STATUS / OCCUPATION

The following questions refer to your current main job, or (if you are not working now) to your last main job. Please complete for both yourself (I) and for your spouse/ex-spouse or long-term partner (II)

(I) Yourself (Please tick one box only per question)

1. What is (was) your employment status?

- Employee ☐
- Self-employed with employees ☐
- Self-employed / freelance without employees (go to **question 4**) ☐
- Housewife (go to **question 4**) ☐

No previous paid employment (excluding housewife) ☐
(go to **question 4**)

2. Number of employees

For employees: indicate below how many people work (worked) for your employer at the place where you work (worked). Then go to question 3.
For self-employed: indicate below how many people you employ (employed). Then go to question 4.

1 to 24 ☐ 25 or more ☐

3. Do (did) you supervise any other employees?

A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis

Yes ☐ No ☐

4. Please tick one box to show which **best** describes the sort of work you do.

(If you are not working now, please tick a box to show what you did in your last job).
PLEASE TICK **ONE BOX ONLY**

Modern professional occupations
such as: teacher - nurse - physiotherapist - social worker - welfare officer - artist - musician - police officer (sergeant or above) - software designer ☐

Clerical and intermediate occupations
such as: secretary - personal assistant - clerical worker - office clerk - call centre agent - nursing auxiliary - nursery nurse ☐

Senior managers or administrators)
such as: finance manager - chief executive ☐

Technical and craft occupations
such as: motor mechanic - fitter - inspector - plumber - printer - tool maker - electrician - gardener - train driver ☐

Semi-routine manual and service occupations
such as: postal worker - machine operative - security guard - caretaker - farm worker - catering assistant - receptionist - sales assistant ☐

Routine manual and service occupations
such as: HGV driver - van driver - cleaner - porter - packer - sewing machinist - messenger - labourer - waiter / waitress - bar staff ☐

Middle or junior managers
such as: office manager - retail manager - bank manager - restaurant manager - warehouse manager - publican ☐

Traditional professional occupations
such as: accountant - solicitor - medical practitioner - scientist - civil / mechanical engineer ☐

EMPLOYMENT STATUS / OCCUPATION (cont.)

(II) Your spouse/ex-spouse/long term partner (Please tick one box only per question)

5. What is (was) his (her) employment status?

- Employee ☐
- Self-employed with employees ☐
- Self-employed / freelance without employees (go to **question 8**) ☐
- Housewife (go to **question 8**) ☐
- No previous paid employment (excluding housewife) (go to **question 8**) ☐

6. Number of employees

For employees: indicate below how many people work (worked) for his/her employer at the place where he/she work (worked). Then go to question 7.
For self-employed: indicate below how many people he/she employs (employed). Then go to question 8.

1 to 24 ☐ 25 or more ☐

7. Do (did) he/she supervise any other employees?

A supervisor or foreman is responsible for overseeing the work of other employees on a day-to-day basis

No ☐ Yes ☐

8. Please tick one box to show which **best** describes the sort of work he/she does.

(If not working now, please tick a box to show what he/she did in his/her last job).

PLEASE TICK **ONE BOX ONLY**

Modern professional occupations
such as: teacher - nurse - physiotherapist - social worker - welfare officer - artist - musician - police officer (sergeant or above) - software designer ☐

Clerical and intermediate occupations
such as: secretary - personal assistant - clerical worker - office clerk - call centre agent - nursing auxiliary - nursery nurse ☐

Senior managers or administrators
(usually responsible for planning, organising and co-ordinating work and for finance)
such as: finance manager - chief executive ☐

Technical and craft occupations
such as: motor mechanic - fitter - inspector - plumber - printer - tool maker - electrician - gardener - train driver ☐

Semi-routine manual and service occupations
such as: postal worker - machine operative - security guard - caretaker - farm worker - catering assistant - receptionist - sales assistant ☐

Routine manual and service occupations
such as: HGV driver - van driver - cleaner - porter - packer - sewing machinist - messenger - labourer - waiter / waitress - bar staff ☐

Middle or junior managers
such as: office manager - retail manager - bank manager - restaurant manager - warehouse manager - publican ☐

Traditional professional occupations
such as: accountant - solicitor - medical practitioner - scientist - civil / mechanical engineer ☐

The Edinburgh Type 2 Diabetes Study
Co-ordinating Centre Public Health Science University of Edinburgh
Teviot Place Edinburgh EH8 9AG Tel/Fax 0131 XXX XXX

Hypoglycaemia Study

Information Sheet

What is the purpose of this part of the Edinburgh Type 2 Diabetes Study?

We would like to know more about your experience of 'severe' hypoglycaemia (hypo) and what kind of help you needed during hypos. It will improve our understanding of hypoglycaemia among people with type 2 diabetes if you complete the attached forms and send them back to us according to the instructions below.

What is a 'severe' hypo?

Hypoglycaemia (low blood sugar) can occur in people treated with diets, tablets and/or insulin. Common symptoms include shakiness, pounding heartbeat, sweating and/or hunger. If the hypo is not treated, then some people can develop dizziness, blurred vision or confusion. A hypo may be treated by eating or drinking something sugary or by medical treatment (typically an injection). We are interested in '**severe'** episodes of hypoglycaemia (severe hypos). This is defined as a hypo in which *you require treatment to be administered by another person* (e.g., family member, friend or health care worker) in order to recover from the hypo, because you are not well enough to treat yourself during that hypo.

Instructions

1. If you have a severe hypo (in which you require help from someone else to recover) in the next 6 months, please either

    a. Complete one of the **white** forms in this slip-case and return it to us in one of the pre-paid envelopes provided

    OR

    b. Call us on (0131) 650 3242 and leave your telephone number with a short message and we will call you back to ask you about your hypo.

2. Even if you do not have a severe hypo over the next 6 months, we would still like to hear from you. Therefore, please complete and

return one of the **yellow** forms in this slipcase to us every 2 months for the next 6 months **whether or not you have a severe hypo.** The month in which we would like to receive each of these yellow forms is given at the top of the form.

Remember, we would like to receive a <u>yellow</u> form from you every two months, whether or not you have a severe hypo.

However, do not worry if you forget to send in a form during the specified month – if we do not receive a form then we will telephone you to check whether you have had a 'severe' hypo

Thank you very much for your help.

White Form

Please complete this form if you have a 'severe' hypo during the 6 months after your last Edinburgh Type 2 Diabetes Study clinic appointment. Please return it to us in one of the pre-paid envelopes provided as soon as possible after the hypo

Please complete the following

1. Name…………………………………….

Contact telephone number……………………………………….

2. When did you have the severe hypo when you needed someone else to treat you *because you were not well enough to treat yourself* during that hypo?

…..…/…..…/……..
(please enter day/month/year)

…… am OR …….pm
(please enter approximate time of day)

3. Who helped you to recover from your severe hypo? **(please tick <u>all</u> that apply)**

Family member/carer/friend ☐

Your own doctor/GP ☐

Ambulance crew ☐

Hospital staff ☐

(please state hospital attended)…………………………………….

Other ☐

(please specify)…………………………..

4. How did you recover from the severe hypo? **(please tick <u>all</u> that apply)**

By taking a sugary drink/food ☐

By having an injection of glucagon ☐

By having an injection of sugary water (glucose) into a vein ☐

Other ☐
(please specify) …………………………............

Don't know ☐

5. Did you take a blood glucose reading during the hypo?

YES ☐ NO ☐

If **YES**

a. Please enter your blood glucose reading:
……………..mmol/l

b. Was this reading taken before or after the treatment?

BEFORE ☐ AFTER ☐

Yellow Form

Please complete and return this form, <u>whether or not you have had a ‘severe’ hypo</u> over the past 2 months

Please complete the following

1. Name…………………………………

Contact telephone number ……………………………………………

2. Have you had a ‘severe’ hypo (requiring help from someone else to recover from the hypo, such as a family member, friend or health care worker) over the past 2 months?

☐ YES (⇒ Continue to Question 3)

☐ NO (⇒ Go to end of form)

3. Have you already informed the research office about this/these hypo(s)

☐ YES (⇒ Go to end of form)

☐ NO (⇒ Question 4)

4. When did you have the severe hypo when you needed someone else to treat you *because you were not well enough to treat yourself* during that hypo?
…..…/…..…/……..
(please enter day/month/year)

…… am OR …….pm
(please enter approximate time of day)

5. Who helped you to recover from your severe hypo? **(please tick <u>all</u> that apply)**

Family member/carer/friend ☐

Your own doctor/GP ☐

Ambulance crew ☐

Hospital staff ☐
(Please state hospital attended)
………………………………………………

Other ☐
(Please specify)……………………………

6. How did you recover from the severe hypo? **(please tick <u>all</u> that apply)**

By taking a sugary drink/food ☐

By having an injection of glucagon ☐

By having an injection of sugary water (glucose) into a vein ☐

Other ☐
(please specify)
…………………………...

Don’t know ☐

7. Did you take a blood glucose reading during the hypo?

YES ☐ NO ☐

If **YES**

a. Please enter your blood glucose reading:
……………..mmol/l

b. Was this reading taken before or after the treatment?

BEFORE ☐ AFTER ☐

Appendix I: Publications made on the basis of this thesis, with permission from Diabetes Care®

Association of N-Terminal Pro-Brain Natriuretic Peptide with Cognitive Function and Depression in Elderly People with Type 2 Diabetes

Insa Feinkohl[1]*, Naveed Sattar[2], Paul Welsh[2], Rebecca M. Reynolds[3], Ian J. Deary[4,5], Mark W. J. Strachan[6], Jackie F. Price[1]* on behalf of the Edinburgh Type 2 Diabetes Study (ET2DS) Investigators

1 Centre for Population Health Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom, **2** Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, Scotland, United Kingdom, **3** Centre for Cardiovascular Sciences, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Scotland, United Kingdom, **4** Psychology in the School of Philosophy, Psychology and Language Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom, **5** Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, Scotland, United Kingdom, **6** Metabolic Unit, Western General Hospital, Edinburgh, Scotland, United Kingdom

Abstract

Background: Type 2 diabetes mellitus is associated with risk of congestive heart failure (CHF), cognitive dysfunction and depression. CHF itself is linked both to poor cognition and depression. The ventricular N-terminal pro-brain natriuretic peptide (NT-proBNP) is a marker of CHF, suggesting potential as a marker for cognitive impairment and/or depression. This was tested in the Edinburgh Type 2 Diabetes Study (ET2DS).

Methodology and Principal Findings: Cross-sectional analysis of 1066 men and women aged 60–75 with type 2 diabetes. Results from seven neuropsychological tests were combined in a standardised general cognitive ability factor, 'g'. A vocabulary-based test estimated pre-morbid cognitive ability. The Hospital Anxiety and Depression Scale (HADS) assessed possible depression. After adjustment for age and sex, raised plasma NT-proBNP was weakly associated with lower 'g' and higher depression scores (ß −0.09, 95% CI −0.13 to −0.03, p = 0.004 and ß 0.08, 95% CI 0.04 to 0.12, p<0.001, respectively). Comparing extreme quintiles of NT-proBNP, subjects in the highest quintile were more likely to have reduced cognitive ability (within the lowest tertile of 'g') and 'possible' depression (HADS depression ≥8) (OR 1.80; 95% CI: 1.20, 2.70; p = 0.005 and OR 2.18; 95% CI: 1.28, 3.71; p = 0.004, respectively). Associations persisted when pre-morbid ability was adjusted for, but as expected were no longer statistically significant following the adjustment for diabetes-related and vascular co-variates (β −0.02, 95% CI −0.07 to 0.03, p>0.05 for 'g'; β 0.03, 95% CI −0.02 to 0.07, p>0.05 for depression scores).

Conclusion: Raised plasma NT-proBNP was weakly but statistically significantly associated with poorer cognitive function and depression. The prospective phases of the ET2DS will help determine whether or not NT-proBNP can be considered a risk marker for subsequent cognitive impairment and incident depression and whether it provides additional information over and above traditional risk factors for these conditions.

Citation: Feinkohl I, Sattar N, Welsh P, Reynolds RM, Deary IJ, et al. (2012) Association of N-Terminal Pro-Brain Natriuretic Peptide with Cognitive Function and Depression in Elderly People with Type 2 Diabetes. PLoS ONE 7(9): e44569. doi:10.1371/journal.pone.0044569

Editor: Christian Herder, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Duesseldorf, Germany

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Competing Interests: The ET2DS is co-funded by Pfizer plc. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

* E-mail: I.Feinkohl@sms.ed.ac.uk (IF); Jackie.Price@ed.ac.uk (JFP)

Introduction

People with type 2 diabetes are at around 1.5 to 2.5-fold increased risk of developing dementia [1,2], a disorder involving progressive cognitive, behavioural and motor deficits. Type 2 diabetes is also associated with an increased risk of age-associated cognitive decline, short of frank dementia [3], and with depression [4,5], a condition closely related to cognitive dysfunction. The underlying mechanisms responsible for these links are unclear.

Co-morbidity with congestive heart failure (CHF) is also prevalent in diabetes [6,7]; there appears to be a poorly defined bidirectional relationship between cardiac function and glucose metabolism. Cardiac stress initiates secretion of natriuretic peptides from the ventricles [8], including N-terminal pro-brain natriuretic peptide (NT-proBNP) the inactive metabolite of the proBNP hormone. Higher circulating NT-proBNP concentrations in populations with diabetes compared with healthy adults have been reported [9,10].

There is some evidence in predominantly non-diabetic populations to suggest that BNP (with which circulating NT-proBNP is highly correlated [11]) is associated with poor cognitive ability. Raised natriuretic peptide concentrations have been related to low performance on measures screening for poor global cognitive functioning potentially indicative of dementia [12–14]. Raised natriuretic peptide concentrations are also found in cognitively impaired individuals [15,16] and may correlate with severity of dementia [14,17]. In the only prospective investigation to date, proBNP predicted dementia at 5-year follow-up [18].

Evidence also indicates raised NT-proBNP in individuals with major depressive disorder (MDD) and correlations between NT-proBNP and number of depressive symptoms in healthy populations [19] and in those with cardiovascular disease [20–22]. Further, left ventricular dysfunction has been associated with poor cognition [23–25] and depression [25]. However, overall evidence is inconclusive, with several studies failing to find a statistically significant association of NT-proBNP with depression or quality of life [26–29], with cognitive function [30,31], or dementia [15].

Given these findings, NT-proBNP could function as a biomarker of accelerated cognitive aging and depression in people with type 2 diabetes. High prevalence of cognitive and mood disorders in type 2 diabetes offers an interesting and novel opportunity to study the relationship between natriuretic peptides, acting as a proxy for cardiac stress, and cognitive function and depression. The present study aims to assess the association of NT-proBNP with level of cognitive ability, with level of ability relative to estimated peak pre-morbid ability, and with depressive symptoms, in a large, representative cohort of elderly patients with the full spectrum of severity of type 2 diabetes (the Edinburgh Type 2 Diabetes Study, ET2DS).

Methods

Study Population

Data from the cross-sectional, baseline phase of the prospective ET2DS were analysed. Details of the recruitment and examination for the ET2DS have been reported previously [32]. In brief, in 2006/7, 1066 community-dwelling men and women with type 2 diabetes mellitus, aged 60 to 75 years and living in the Lothian area of Scotland, UK, were selected at random from a comprehensive register of patients with diabetes attending primary or secondary care in this region. Subjects underwent detailed physical and cognitive examinations in a dedicated research clinic by specially trained nurses using standard operating procedures.

Ethics Statement

Assessments complied with the ethical rules of the Declaration of Helsinki. Ethical approval for the study was obtained from the Lothian Medical Research Ethics Committee, and all participants gave written informed consent.

Physical Examination

Participants were assessed by self-completion questionnaire and physical examination for a range of demographic and clinical characteristics. Diabetes-related measurements included plasma HbA1c, fasting glucose, duration of diabetes and mode of treatment. Vascular measurements included systolic and diastolic brachial blood pressure, body mass index (BMI), waist-hip ratio (WHR), % body fat, smoking status, alcohol intake, total serum cholesterol and ankle brachial index (ABI). ABI is the ratio of systolic blood pressure in the ankle to the systolic blood pressure in the arm, and has been used as a measure both of peripheral arterial disease and more generalised subclinical atherosclerosis [33,34]. Pre-specified criteria were used to define prior myocardial infarction (MI), angina or stroke using a combination of self-report of a doctor diagnosis, WHO chest pain questionnaire, 12-lead ECG and linkage to hospital discharge records, as has been described in detail previously [35].

Cognitive and Mood Assessment

Seven neuropsychological tests measured various dimensions of cognitive ability. The Borkowski Verbal Fluency Test (BVFT) examined executive function. Immediate and delayed recall was assessed in the Logical Memory subtest (LM) of the Wechsler Memory Scale 3rd Edition (WMS-III). The Faces subtest of the WMS-III was a measure of non-verbal memory. Mental flexibility and visual attention were examined by the Trail-Making Test B (TMT-B). Several subtests of the Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) were administered: Digit Symbol Coding (DSC; speed of information processing), Letter Number Sequencing (LNS; working memory) and the Matrix Reasoning test (MR; non-verbal reasoning). Vocabulary ('crystallised' intelligence) was measured using the combined junior and senior Mill Hill Vocabulary scale (MHVS) synonyms. As the results of vocabulary tests show very little mean decline with ageing, they can be used to estimate peak, prior cognitive ability [36]. When late-life cognitive ability scores are adjusted for vocabulary-based test scores, the residuals correlate highly with actual measures of cognitive change over a lifetime [37]. A score of below 24 out of 30 on the Mini-Mental-State Examination (MMSE; [38]) indicated presence of possible dementia. Presence of depression and anxiety was assessed on the Hospital Anxiety and Depression Scale (HADS; [39]). Responses were made on four-point scales, with maximum scores of 21 on each of two subscales, the HADS-A and HADS-D. In addition to the use of the HADS-D as a continuous outcome, 'possible cases' of clinical depression (HADS-D $\geq$8) were identified. Both these applications of the HADS are widely employed in the literature [40], and the cut-point of 8 has been shown to have high sensitivity and specificity [41]. A majority of participants completed the entire cognitive test battery and mood assessment ($n = 1048$ to 1065 for individual tests), and provided data on all physical measures ($n = 1028$ to 1066). Incompleteness was mainly due to physical difficulty rather than test refusal.

Measurement of NT-proBNP

Fasting blood samples were taken at the research clinic, and plasma was frozen for storage. Plasma NT-proBNP concentrations were determined using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK) calibrated using the manufacturer's reagents. Manufacturer's controls were used with limits of acceptability defined by the manufacturer. Low control CV was 6.7% and high control CV was 4.9%.

Statistical Methods

Distribution of values was skewed for NT-proBNP, HADS-D, and TMT-B. These variables were transformed to their natural logarithm values.

Individuals who perform well on one cognitive test tend to perform well on another, so that different cognitive tests load on a single common factor, '*g*' [42]. Principal component analysis revealed that all seven cognitive tests (LM, Faces, MR, DSC, TMT-B, LNS, BVFT) could indeed be captured by the single standardised factor, '*g*'.

Initial two-tailed Pearson correlations tested associations of NT-proBNP with other clinical measures, cognitive outcome and HADS depression scores. Mean NT-proBNP levels were compared between groups with suspected dementia (MMSE$<$24), and between those with suspected clinical depression (HADS-D score $\geq$8) and those without.

In exploratory univariate analyses, mean '*g*' and HADS depression scores were compared between the highest and the lower four quintile NT-proBNP groups. The likelihood of low cognitive functioning (scoring in the lowest versus intermediate or highest tertiles of '*g*') and of suspected clinical depression were calculated for the highest quintile NT-proBNP as an example of a group with raised NT-proBNP levels.

Scores on the different cognitive tests were also treated as individual continuous outcome variables. NT-proBNP was modelled on each of these, '*g*', and HADS depression, adjusting for potential confounders in staged and cumulative linear regression analyses. Confounders were selected on the basis of results from exploratory univariate analyses and reports in the literature of confounding or mediating roles in the relationship of NT-proBNP with cognition or depression. Results from the regression analyses were presented as β regression coefficients to show the direction and strength of the associations, signifying the change in cognitive and depression scores for each unit increase in NT-proBNP.

For cognitive outcome variables, only age and sex were controlled for in a first model. Estimated pre-morbid cognitive ability (MHVS) was entered in a second stage, before the addition of diabetes-related and cardiovascular risk factor variables (smoking status, blood pressure, BMI, HbA1c, cholesterol and mode of treatment). MI, angina, stroke and ankle brachial index (ABI) were then entered, but had not previously been included in order to avoid over-adjustment. HADS depression scores were added in a final model in order to determine the potential independence of NT-proBNP associations with cognition and depression. The first two modelling steps were also followed for the HADS depression outcome. In a third step, HbA1c and mode of treatment were controlled for. Cardiovascular disease variables were only added into the model in a final step, again in order to avoid over-adjustment given the role of NT-proBNP as a marker of cardiovascular disease. Analyses were performed using SPSS for Windows, version 14.0 (IBM Corporation, New York).

Results

Cohort characteristics and classical risk factors

Characteristics of the ET2DS participants are shown in Table 1. The study population (n = 1066) has been shown previously to be largely representative of all people invited to participate in the study (n = 5454) and therefore of the target population of older men and women with type 2 diabetes living in the general population [35]. Mean age was 67.9 years. Mean plasma NT-proBNP levels were similar in men and women (geometric means 79 pg/ml ±3 vs 84 pg/ml ±3; $p = 0.41$ and correlated positively with age ($r = 0.25$; $p<0.001$). Controlling for age and sex, NT-proBNP was significantly associated with the following variables: duration of diabetes ($r = 0.14$; $p<0.001$), diastolic blood pressure ($r = -0.09$; $p = 0.008$), ABI ($r = -0.14$; $p<0.001$), BMI ($r = 0.11$; $p<0.001$) and cholesterol ($r = -0.10$; $p = 0.005$), but not with HbA1c, plasma glucose or systolic blood pressure ($p>0.05$). Mean levels differed between individuals with MI, stroke or angina and those without ($p<0.001$ respectively), but not between smokers and non-smokers ($p>0.05$).

NT-proBNP associations with cognitive performance

After controlling for age and sex, NT-proBNP was weakly but statistically significantly correlated with decreased performance on MHVS, Logical Memory, TMT-B, DSC, MR, the general ability factor '*g*' and HADS depression. NT-proBNP levels accounted for <1% of variance in cognitive test scores and '*g*' ($r^2 = 0.005$ to 0.008), and for 1.7% of depression scores. ($r^2 = 0.017$; Table 2). NT-proBNP was not significantly different in the small group of participants with possible dementia (MMSE<24, n = 30) compared with higher scoring individuals (geometric mean 106 pg/ml ±3 vs 81 pg/ml ±3, $p = 0.24$). However, the group with possible clinical depression (HADS depression score ≥8, n = 78) had lower mean MMSE scores (geometric means 27.80±1.08 vs 28.28±1.08, $p = 0.052$, Cohen's $d = 0.24$) and higher NT-proBNP levels (geometric mean 107 pg/ml ±3 vs 80 pg/ml ±3, $p = 0.03$, Cohen's $d = 0.28$) compared to the non-depressed group.

To further explore whether plasma NT-proBNP could be a marker of reduced cognitive function irrespective of other possible markers, we examined differences in cognitive ability across quintiles of NT-proBNP (geometric cutpoints were 29.70, 59.15, 100.48 and 217.02 pg/ml). An analysis of covariance with adjustment for age and sex suggested an overall trend across quintiles (adjusted mean 'g' = 0.02, 0.14, 0.13, −0.09 and −0.18 respectively; $p = 0.003$). Subjects in the highest quintile of NT-proBNP (n = 199) had lower 'g' than those in the remaining four quintiles (n = 807; adjusted means −0.18 versus 0.05; 95% CI for difference −0.39, −0.08; $p = 0.003$). In unadjusted analyses comparing the highest quintile of NT-proBNP to all other quintiles, the odds ratios for low cognitive ability ('g' in the lowest tertile) was 2.14 (95% CI: 1.44, 3.18; $p<0.001$). After adjustment for age and sex, the association was marginally attenuated to 1.80 (95% CI: 1.20, 2.70; $p = 0.005$).

For 'g' and each of the constituent cognitive tests, multiple regression analyses were carried out (Table 3). The initial model controlled for age and sex. Associations with NT-proBNP reached statistical significance for a number of cognitive tests (Logical Memory, TMT-B, DSC, MR), and 'g'. In a second step, pre-morbid cognitive ability (MHVS) was added into the model. The associations with TMT-B, DSC, MR, and 'g' remained statistically significant, and for Logical Memory approached significance. When a range of potential confounding and/or mediating variables, including conventional cardiovascular risk factors together with HbA1c and treatment type were added in a third model, only the association with Logical Memory remained statistically significant and also survived the further adjustment for previous cardiovascular disease and HADS depression in a final step.

NT-proBNP associations with depression

Adjusting for age and sex, HADS depression scores gradually increased across NT-proBNP quintiles (geometric mean scores were 2.77, 2.94, 3.19, 3.25 and 3.56 respectively; $p = 0.007$). The highest NT-proBNP quintile had higher mean depression scores compared with the lower four quintiles (95% CI 0.05, 0.28; $p = 0.006$). The age- and sex-adjusted odds ratio (OR) for possible clinical depression, comparing the highest versus the lower four quintiles, was 2.18 (95% CI 1.28, 3.71; $p = 0.004$).

Multivariable modelling was repeated for HADS depression. The age and sex adjusted association reached significance and survived additional adjustment for pre-morbid cognitive ability, as well as diabetes-associated variables. The association became non-significant following the addition of previous cardiovascular disease into the model (Table 4).

Discussion

This epidemiological study investigated the contribution of NT-proBNP levels to performance on a large battery of cognitive tests and symptoms of depression in older people with type 2 diabetes using one of the largest community-based study populations on this topic. Cognitive ability, measured by the standardised factor '*g*', was lower, and depression higher, in patients with raised plasma NT-proBNP. Participants with peptide levels in the highest quintile of NT-proBNP distribution were almost two times more likely to have reduced cognitive ability (lowest tertile of '*g*') and

Table 1. Baseline characteristics of the ET2DS population.

Variable	Mean ± SD, median (quartile range) or n (%)
Age (years)	67.9±4.2
Male sex (%)	547 (51.3)
Duration of diabetes (years)	6 (3–11)
Treatment of diabetes with insulin +/− tablets	186 (17)
Treatment with tablets alone	679 (64)
Treatment with dietary change alone	200 (19)
HbA1c (%)	7.4±1.1
Fasting plasma glucose (mmol/L)	7.6±2.1
Systolic BP (mmHg)	133±16
Diastolic BP (mmHg)	69±9
Myocardial infarction	150 (14)
Angina	298 (28)
Stroke	62 (6)
ABI	0.98±0.21
BMI (kg/m^2)	31.4±5.7
Total cholesterol (mmol/L)	4.3±0.9
Smoking (current) (%)	153 (14.4)
NT-proBNP (pg/ml)	75 (37–169)
Hospital Anxiety and Depression Questionnaire (HADS): Depression score	3 (1–6)
HADS depression score ≥8	78 (7.3)
Mini-Mental State Examination <24/30 (%)	30 (2.9)
Trail-Making Test B	104 (81–138)
Matrix Reasoning	12.8±5.3
Digit Symbol Coding	49.2±14.8
Verbal Fluency Test	36.9±12.8
Letter Number Sequencing	9.7±2.8
Faces	65.8±7.9
Logical Memory	25.2±8.2
g	0.00±1.00
Mill Hill Vocabulary Scale	30.9±5.2

Mean ± SD is given for normally distributed variables.
Median (quartile range) is given for non-normally distributed variables.
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were more than two times more likely to have possible clinical depression following the adjustment for age and sex, compared with the remaining population, but associations of NT-proBNP with '*g*' and depression scores no longer reached statistical significance when diabetes-related and vascular co-variates were additionally controlled for.

Although not the main focus of the present investigation, raised NT-proBNP was also found to be associated with poorer pre-morbid cognitive ability. This finding could cast doubt over a causal relationship between cardiac stress (as measured by natriuretic peptides) and late-life cognitive ability in people with diabetes, since 'reverse causation' and confounding are possible explanations [43]. Low early life cognitive ability leads to lower late-life ability, and exposes individuals to poor lifestyle choices, health problems with associated raised biomarker levels, and earlier death [44]. However, the reported weak associations between NT-proBNP and current cognitive ability remained significant when pre-morbid ability was controlled for.

The association between reduced late-life cognitive ability and plasma NT-proBNP is consistent with previous studies on predominantly non-diabetic populations [12–14]. However, previous studies have predominantly used simple screening instruments, such as the MMSE, to measure cognitive function. Our use of a general ability factor derived from a number of different cognitive tests is unique in the literature on NT-proBNP and cognition. Although the age- and sex-adjusted odds of reduced cognitive ability for the high NT-proBNP group were comparable to those reported by Daniels et al. [12], the multivariate associations between NT-proBNP and cognitive scores were relatively weak compared with some previous investigations [13,14]. Peptide levels were also similar in subjects with and without suspected dementia determined using the MMSE (MMSE<24) compared with others. This finding is consistent with previous studies [15,30,31], but may reflect flaws in the use of the MMSE, designed to screen for dementia, as a measure of milder forms of cognitive impairment [45].

Table 2. Age and sex-adjusted two- tailed partial correlations of NT-proBNP with cognitive and depression scores.

	Partial correlation coefficient	p-value
Mill-Hill Vocabulary Scale	−0.07	0.042
Borkowski Verbal Fluency	<0.01	0.963
Logical Memory	−0.08	0.015
Faces	−0.05	0.139
ln(Trail-Making-Test-B)	0.08	0.012
Digit Symbol Coding	−0.09	0.006
Letter Number Sequencing	−0.05	0.146
Matrix Reasoning	−0.08	0.010
g	−0.09	0.007
ln(HADS depression)	0.13	<0.001

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Associations between NT-proBNP and deficits in specific cognitive domains were also explored. When age and sex were controlled for, peptide levels were unrelated to performance on BVFT, Faces or LNS, but were associated with poor performance on Logical Memory, TMT-B, DSC and MR. Furthermore, these relatively weak associations (except that for Logical Memory, for which a trend was observed) remained statistically significant following adjustment for estimated pre-morbid ability. The finding of inverse associations between peptide levels and performance on the TMT-B test of visual attention and the Matrix Reasoning test of non-verbal reasoning is consistent with investigations in non-diabetic populations [12,14]. NT-proBNP was unrelated to verbal fluency (BVFT) both in the present study and in Gunstad et al. [14]. Associations in Daniels et al. [12] also only reached statistical significance when analyses were largely unadjusted. NT-proBNP has not previously been investigated in relation to nonverbal memory (Faces), speed of information processing (DSC) or working memory (LNS), of which we found an association only with speed of processing. It must be noted that since a large number of analyses were carried out, it is possible that the statistically significant associations arose through type I error. Further studies are required to confirm or refute these findings.

For any disease, the identification of biomarkers indicating increased risk of complications and co-morbidities is vital to allow identification of high risk individuals. Despite high prevalence of vascular disease and associated high average NT-proBNP levels in people with type 2 diabetes [9,10], as well as high prevalences of cognitive and mood disorders, to our knowledge, this is the first investigation to focus on the relationship of NT-proBNP with cognitive function and mood in type 2 diabetes.

The association of natriuretic peptides with late cognitive ability may well reflect underlying cardiac stress and associated vascular disease, rather than any direct causal relationship. This is supported by the attenuation of the relationship between cognitive ability and NT-proBNP following the adjustment for a range of cardiovascular risk factors and cardiovascular disease, which rendered it statistically non-significant. A weak association between NT-proBNP and the Logical Memory test (which measures verbal declarative memory through recall of a short story and is particularly sensitive to progression of type 2 diabetes [46]) was unique, in that it was not attenuated by the adjustment for cardiovascular or diabetes-associated variables, or HADS depression scores. Despite the possibility that the result represents a type I error, it is an intriguing finding which merits further investigation.

Raised NT-proBNP was also associated with high depression scores, supporting previous findings from non-diabetic populations [19–22]. The relative weakness of the associations is consistent with one previous report [22], although associations overall tend to be larger [19–21]. Despite predicting high risk of future vascular disease and depression, low pre-morbid ability did not confound the association. Pathways linking depression and NT-proBNP levels have been suggested previously [19]. We found evidence

Table 3. Multivariate associations between NT-proBNP and cognitive test scores.

		MHVS	g	BVFT	LM	FACES	ln(TMT-B)	DSC	LNS	MR
Model 1a	β	−0.26	−0.09	0.02	−0.49	−0.31	0.03	−0.96	−0.10	−0.35
	95% CI	−0.54, 0.01	−0.13, −0.03**	−0.65, 0.69	−0.91, −0.06*	−0.71, 0.10	0.01, 0.05*	−1.71, −0.20**	−0.25, 0.04	−0.62, −0.08**
Model 2b	β		−0.05	0.28	−0.36	−0.20	0.02	−0.69	−0.04	−0.25
	95% CI		−0.09, 0.00*	−0.33, −0.89	−0.75, 0.04	−0.59, 0.19	0.00, 0.04*	−1.38, 0.00*	−0.18, 0.90	−0.50, −0.01*
Model 3c	β		−0.04	0.38	−0.48	−0.15	0.02	−0.26	−0.04	−0.23
	95% CI		−0.08, 0.01	−0.26, 1.01	−0.89, −0.07*	−0.56, 0.26	0.00, 0.04	−0.98, 0.46	−0.17, 0.10	−0.48, 0.02
Model 4d	β		−0.02	0.18	−0.57	−0.01	0.02	0.19	−0.01	−0.15
	95% CI		−0.07, 0.03	−0.52, 0.87	−1.02, −0.11*	−0.46, 0.44	−0.01, 0.04	−0.60, 0.98	−0.16, 0.15	−0.43, 0.13
Model 5e	β		−0.02	0.16	−0.56	−0.03	0.01	0.26	0.01	−0.14
	95% CI		−0.06, 0.03	−0.56, 0.88	−1.03, −0.08*	−0.50, 0.44	−0.01, 0.04	−0.54, 1.07	−0.15, 0.16	−0.42, 0.15

The β scores are regression coefficients.
[a]adjusted for age and sex.
[b]adjusted for +MHVS.
[c]adjusted for + smoking status, blood pressure, BMI, HbA1c, cholesterol, mode of treatment.
[d]adjusted for + MI, angina, stroke, ABI.
[e]adjusted for + ln(HADS depression).
*significant at 0.05 level.
**significant at 0.01 level.
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Table 4. Multivariate associations between NT-proBNP and depression scores.

		ln(HADS depression)
Model 1[a]	**β**	0.08
	95% CI	0.04, 0.12**
Model 2[b]	**β**	0.08
	95% CI	0.04, 0.12**
Model 3[c]	**β**	0.07
	95% CI	0.03, 0.11*
Model 4[d]	**β**	0.03
	95% CI	−0.02, 0.07

The β scores are regression coefficients.
[a]adjusted for age and sex.
[b]adjusted for +MHVS.
[c]adjusted for + HbA1c, mode of treatment.
[d]adjusted for + MI, angina, stroke, ABI.
*significant at 0.05 level.
**significant at 0.01 level.
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against this, as the association became non-significant when previous cardiovascular disease was controlled for. Depression is associated with a harmful lifestyle and subsequent endothelial damage, and also accelerates the progression of cardiac illness through effects on the hypothalamic-pituitary adrenal (HPA) axis, increasing the risk of heart failure [47]. Conversely, heart failure and other chronic illness initiate symptoms of depression, including hypersomnia, fatigue and loss of energy [48], constructs included in the HADS questionnaire.

Overall, the clinical relevance of natriuretic peptides as biomarkers for poor cognitive function and depression is unclear. Despite a relatively weak multivariate association, individuals in the highest NT-proBNP quintile were substantially more likely to have reduced cognitive ability and depression compared with the remaining population. NT-proBNP may function as a biomarker of subclinical cerebrovascular disease particularly in individuals who experience cardiac stress. This extends its well-established role as a predictor of cardiovascular and cerebrovascular events [49]. Mirroring a greater ability of natriuretic peptides compared with other biomarkers, such as c-reactive protein (CRP), to predict these outcomes [50], the association of NT-proBNP with cognitive function in the present study was also stronger than that of CRP with cognitive function in a previous analysis [35].

A strength of the current study was the ability to adjust for a wide variety of variables, both those which are commonly controlled for (e.g., age), and those which may be confounding or mediating factors underlying the relationship between NT-proBNP and cognitive ability (e.g., vascular risk factors; cardiovascular disease). Due to the large size of the study, results are more robust than those of previous investigations carried out on smaller scales [15–17] and the analysis had high power to detect even weak associations, although admittedly, the clinical relevance of such associations is debatable. The latter was reflected in the conservative interpretation of statistically significant results. The cross-sectional nature of the study and the resulting unclear direction of the reported associations is a weakness of the study, although this was partly offset by our adjustment for pre-morbid cognitive ability. The MHVS as a measure of pre-morbid ability is advantageous over the often used level of education, which is subject to self-report bias.

In conclusion, we found some evidence for NT-proBNP as a biomarker of low cognitive ability and depression in elderly people with type 2 diabetes, although the associations were relatively weak and were largely explained by previous cardiovascular disease. The value of NT-proBNP measurement over and above the assessment of traditional markers of cognitive dysfunction and depression remains unclear, and further studies on its relationship with these disorders are needed. Analysis of the second wave of data of the prospective ET2DS will provide an ideal opportunity to investigate the associations of natriuretic peptide levels with risk of subsequent cognitive decline and the development of depression.

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Author Contributions

Conceived and designed the experiments: JFP MWJS. Performed the experiments: IJD NS PW RMR. Analyzed the data: IF. Wrote the paper: IF NS PW RMR IJD MWJS JFP.

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Clinical and Subclinical Macrovascular Disease as Predictors of Cognitive Decline in Older Patients With Type 2 Diabetes

The Edinburgh Type 2 Diabetes Study

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OBJECTIVE—Macrovascular disease may contribute to increased risk of accelerated cognitive decline in patients with type 2 diabetes. We aimed to determine associations of measures of macrovascular disease with cognitive change in a cognitively healthy older population with type 2 diabetes.

RESEARCH DESIGN AND METHODS—Eight hundred thirty-one men and women (aged 60–75 years) attended two waves of the prospective Edinburgh Type 2 Diabetes Study (ET2DS). At baseline, clinical and subclinical macrovascular disease was measured, including cardiovascular event history, carotid intima-media thickness (cIMT), ankle brachial index (ABI), and serum N-terminal probrain natriuretic peptide (NT-proBNP). Seven neuropsychological tests were administered at baseline and after 4 years; scores were combined to a standardized general ability factor (*g*). Adjustment of follow-up *g* for baseline *g* assessed 4-year cognitive change. Adjustment for vocabulary (estimated premorbid ability) was used to estimate lifetime cognitive change.

RESULTS—Measures of cognitive decline were significantly associated with stroke, NT-proBNP, ABI, and cIMT, but not with nonstroke vascular events. The association of stroke with increased estimated lifetime cognitive decline (standardized β, -0.12) and of subclinical markers with actual 4-year decline (standardized β, -0.12, 0.12, and -0.15 for NT-proBNP, ABI, and cIMT, respectively) reached the Bonferroni-adjusted level of statistical significance ($P < 0.006$). Results altered only slightly on adjustment for vascular risk factors.

CONCLUSIONS—Stroke and subclinical markers of cardiac stress and generalized atherosclerosis are associated with cognitive decline in older patients with type 2 diabetes. Further investigation into the potential use of subclinical vascular disease markers in predicting cognitive decline is warranted.

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Cognitive abilities are essential for independent living in later life, and some domains of cognitive functioning decline in mean level from relatively early adulthood (1). Age-related cognitive decline is accompanied by pathological changes in the brain, including cerebral microvascular changes, and although individual differences exist in the severity of age-related microvascular damage in the brain, this is difficult to investigate noninvasively. Systemic atherosclerotic changes in the body may serve as a marker of vascular-related changes in the brain (2) that, in turn, lead to cognitive deficits (3,4). However, the potential of large vessel changes distant from the brain itself to function as markers of cognitive decline remains unclear. We aimed to study a range of measures of clinical and subclinical macrovascular disease that focus on different areas of the vasculature or different underlying pathophysiological mechanisms to assess which of these might function as proxies of cognitive decline.

Understanding the role of macrovascular disease in age-related cognitive impairment is particularly important in diabetes, given the higher prevalence of atherosclerotic large vessel disease as well as the accelerated cognitive decline and increased risk of cognitive impairment (5,6) associated with this condition, and the potentially modifiable nature of macrovascular disease (7). The prevalence of stroke, of transient ischemic attack (TIA) (8), and of coronary heart disease (9) are higher in diabetic populations than in nondiabetic populations, and average natriuretic peptide levels, a marker of cardiac stress, are increased (10). Markers of subclinical atherosclerosis also are altered, with increased average carotid intima-media thickness (cIMT) (11) and reduced mean ankle brachial index (ABI) (12). Despite this, investigation into the role of macrovascular disease in age-related cognitive impairment in people with diabetes is limited compared with investigation into this issue in the general (predominantly nondiabetic) population. We set out to determine the association of a variety of measures of subclinical macrovascular disease and cardiovascular event categories with cognitive decline in a sample of older people, all of whom had diabetes (the Edinburgh Type 2 Diabetes Study [ET2DS]). We did so using two cognitive outcomes, actual late-life cognitive change over a 4-year period and estimated lifetime cognitive change. These analyses are timely given the increasing prevalence of diabetes at younger ages (13) that, together with greater survival (14) and greater lifetime exposure to diabetes in current generations, is likely to contribute to increasing prevalence of cognitive impairment.

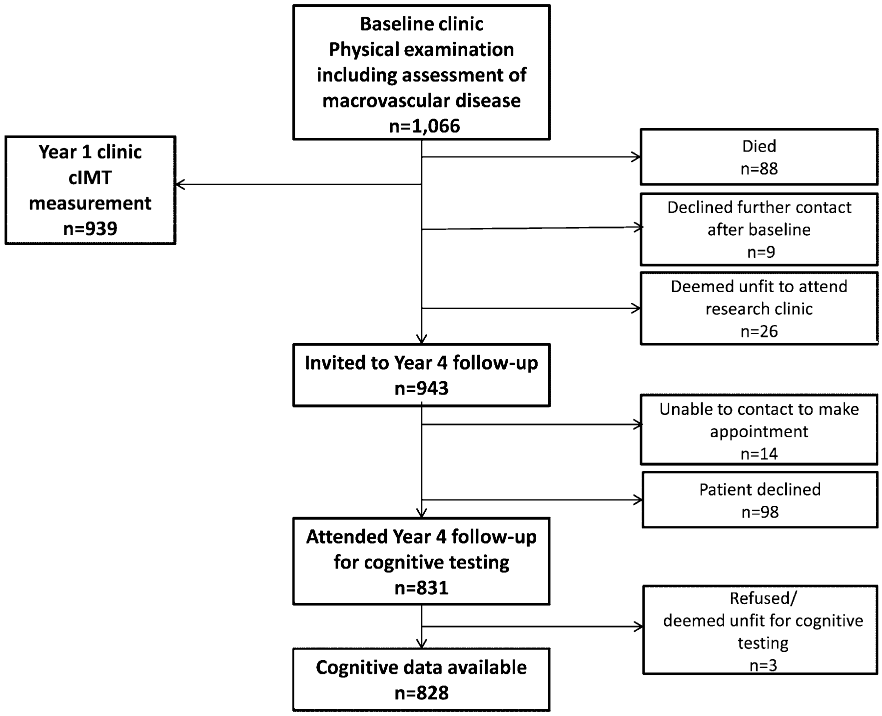

Figure 1—*Subject participation in the ET2DS.*

RESEARCH DESIGN AND METHODS

Study population

In 2006, the ET2DS recruited a randomly selected sample of older adults with type 2 diabetes from the Lothian Diabetes Register, a population-based disease register that contains details of nearly all individuals with diagnosed diabetes living in the Lothian region of Scotland, U.K. The study had ethical approval from the Lothian Medical Research Ethics Committee. Of 5,454 individuals aged 60–74 years who were invited to participate, 1,066 were recruited. Details of recruitment have been described previously (15) and representativeness of the cohort has been demonstrated by comparing demographic and clinical characteristics of responders and nonresponders (16).

At baseline in 2006–2007, all study participants attended a dedicated research clinic for extensive physical and cognitive examination and 939 (88%) returned for further examination after ~1 year (2007–2008). All participants were considered for repeat cognitive testing after 4 years (2010–2011). Between baseline and year 4, 88 participants had died, 9 declined further participation, and 26 were deemed unfit to continue participation. Invitations were sent out to 943 (88%) participants. Of these, 98 declined to attend and 14 could not be contacted; 831 (78%) ultimately attended the year 4 clinic (Fig. 1). Reasons for nonattendance at follow-up included poor health (including dementia), responsibilities as a carer, and loss of interest in participation. Nonattenders were followed-up using subject or general practitioner questionnaires, record linkage to hospital discharge and death certificate data, and review of hospital notes when required. Informed consent was obtained from all participants at each clinic attendance.

Physical and cognitive examination

All assessments were performed by specially trained nurses following standard operating procedures and have been described in detail previously (15). At baseline, fasting blood samples were taken for measurement of total serum cholesterol, HbA_{1c}, glucose, and plasma N-terminal probrain natriuretic peptide (NT-proBNP). Participants completed a questionnaire with standard questions about vascular disease and vascular risk factors, including smoking history, doctor diagnosis of myocardial infarction (MI), angina, and stroke, the World Health Organization chest pain questionnaire, and the Edinburgh Claudication Questionnaire (17).

Height and weight, brachial blood pressure, and a standard 12-lead electrocardiogram were recorded and measurement of right and left brachial, posterior tibial, and dorsalis pedis systolic pressures were used to calculate the ABI. Scottish Morbidity Record (SMR01) data on all medical and surgical discharges from Scottish hospitals between 1981 and 2011 were obtained. Any ICD-10 or ICD-9 codes for cardiovascular or cerebrovascular disease recorded between 1981 and 2007 were extracted and used together with questionnaire data on vascular disease and electrocardiogram findings (as well as review of clinical notes when required) to define MI, angina, stroke, and TIA, as detailed previously (16,18). Peripheral arterial disease was defined as presence of intermittent claudication on the Edinburgh Claudication Questionnaire.

At year 1, carotid IMT was measured bilaterally in three separate images of the common carotid artery, 1 to 2 cm below the bifurcation and in areas free of plaque using a Sonoline Elegra Ultrasound Imaging System (Siemens Medical Systems). Mean cIMT was calculated for the left and right carotid arteries; the higher of the two values was used for analyses.

At baseline and at 4-year follow-up, seven neuropsychological tests were used to measure several domains of cognitive ability. The Borkowski Verbal Fluency Test (BVFT) examined executive function. The Logical Memory (LM) subtest of the Wechsler Memory Scale, third edition, assessed immediate and delayed verbal declarative memory. Nonverbal memory was measured by the Faces subtest of the same scale. The Trail-Making Test B (TMT-B) examined mental flexibility and executive function. The Digit Symbol Coding (DSC) subtest of the Wechsler Adult Intelligence Scale, third edition, measured speed of information processing. The Letter-Number Sequencing and the Matrix Reasoning (MR) subtests from the Wechsler Adult Intelligence Scale, third edition, assessed working memory and nonverbal reasoning, respectively. Scores on the combined junior and senior Mill Hill Vocabulary Scale (MHVS) for synonyms, which correlate strongly with scores on the more traditionally used National Adult Reading Test (19), tested vocabulary abilities. Symptoms of depression and anxiety were self-reported on the Hospital Anxiety and Depression Scale (20). The Mini-Mental-State Examination (MMSE) (21) assessed global cognitive function. A cut-point of $<$24 out of 30 is traditionally used to identify individuals with possible dementia (22). However, because of the low validity and reliability of this method (23), we applied the following additional criteria to define dementia: using medication for dementia at baseline or year 4, or both; dementia coding (F00, F01, F02, F03, or G30) from SMR01 or on death certificate between 1981 and completion of year 4 follow-up in 2011; report of psychiatrist diagnosis of dementia in a questionnaire sent to general practitioners of all subjects with MMSE $<$24 at baseline or follow-up and subjects who did not attend the year 4 clinic; a psychiatrist's diagnosis of dementia obtained from a review of psychiatry or hospital notes performed after completion of year 4 follow-up; and self-reported or relative-reported dementia. Dementia was recorded if two of these criteria were met or, in subjects with MMSE score $<$24 at baseline or follow-up, or missing at follow-up, if one of the first four criteria was met.

Statistical analysis

Preparation of data. Because of skewed distributions, NT-proBNP and TMT-B were transformed to their natural logarithm values. When data for one, two, or three out of the seven cognitive tests were missing, multiple imputation accounting for age and sex was performed. In accordance with previous literature (24), mild cognitive impairment (MCI) was defined as scoring in the lowest fifth percentile of memory scores (LM), with MMSE $\geq$24 and failure to meet criteria for dementia.

Univariate analyses. Exploratory analyses determined associations among baseline predictors, correlations between cognitive scores measured at baseline and at follow-up, and 4-year change in mean cognitive scores. Mean ABI, cIMT, and NT-proBNP and prevalence of macrovascular disease were compared in participants with dementia, in those who had conversion to MCI during follow-up, and in the remaining sample.

Multivariate analyses. Multivariate regression analyses tested for associations between baseline macrovascular disease and follow-up cognitive scores, estimated lifetime, and 4-year cognitive change. To estimate lifetime cognitive change, follow-up cognitive scores were adjusted for baseline MHVS scores. Because vocabulary tests measure "crystallized" intelligence and show little age-related declines, they may be used to estimate peak previous cognitive ability (25). Details of this approach have been published previously (26); essentially, by controlling for a well-validated estimate of peak cognitive ability, it was possible to assess associations of macrovascular disease variables with estimated decline from best-ever level of cognitive function. To measure actual 4-year cognitive change, follow-up scores were adjusted for baseline scores on the respective cognitive test.

General ability factor g. Principal components analysis was performed on cognitive test scores with extraction of components with Eigen values $>$1 to function as additional cognitive outcomes. A single component of general cognitive ability (usually referred to as *g*) was determined for follow-up (factor loading: Faces, 0.51; LM, 0.61; DSC, 0.62; BVFT, 0.62; MR, 0.69; Letter-Number Sequencing, 0.75; DSC and TMT-B, 0.81), which accounted for 47.4% of the total variance. Strictly speaking, principal components analysis does not extract "factors," but the usage is common and we adopted it here. It has been found that individuals who perform well on one cognitive test tend to perform well on another (27). Adjustment of follow-up *g* for baseline *g* to signify 4-year cognitive change requires that both are standardized in the same population. To achieve this, cognitive data were arranged in the statistical program so that each of seven columns included cognitive scores obtained at baseline and at follow-up, a single principal components analysis was performed, and saved regression scores of the first principal component were separated into two columns according to time point. The resulting baseline *g* and follow-up *g*, which therefore have identical factor loading (Faces, 0.43; LM, 0.55; BVFT, 0.65; MR, 0.67, Letter-Number Sequencing, 0.72; DSC, 0.75; TMT-B, 0.79), were then used for all analyses of 4-year change in *g*.

Covariates and correction for multiple comparisons. Linear regression analyses were further adjusted for conventional cardiovascular risk factors (brachial blood pressure, serum total cholesterol, and history of cigarette smoking). Post hoc analyses controlling for baseline MHVS determined the independence of associations with 4-year cognitive change from premorbid ability. To counteract risk of type I error, Bonferroni corrections are commonly applied to the cut-point for statistical significance, but this increases the risk of accepting incorrect null hypotheses (type II error). To balance these risks, and because all three cognitive outcomes were derived from one variable, we applied a Bonferroni correction on the basis of nine individual analyses only (to reflect nine predictor variables; cut-point $P < 0.006$). Analyses were performed using SPSS for Windows version 19.0 (IBM).

RESULTS—A comparison of baseline sociodemographic and physical characteristics revealed modest differences between participants who returned for year 4 follow-up ("attenders") and those who did not ("nonattenders") (Table 1). Attenders had significantly better cognitive ability at baseline and were less likely to have dementia by year 4 (n = 4, 0.5%) compared with nonattenders (n = 15, 6.4%). Further analyses were performed on data from attenders; in this group, mean age at baseline was 67.7 years and 293 participants (35.3%) had had at least one cardiovascular event (stroke, TIA, MI, angina, or intermittent claudication).

Many measures of macrovascular disease were associated with each other; participants with low ABI tended to have higher cIMT (r = $-$0.10; P = 0.004) and increased plasma NT-proBNP levels (r = $-$0.16; $P < 0.001$). NT-proBNP correlated positively with cIMT (r = 0.13; $P < 0.001$). Participants with history of any cardiovascular disease (CVD), stroke, or angina all had significantly higher mean NT-proBNP and cIMT, and lower mean ABI compared with the remaining population (all associations $P < 0.001$ for any CVD; $P < 0.05$

Table 1—*Baseline characteristics of ET2DS participants attending and not attending 4-year follow-up for cognitive testing*

	Attenders		Nonattenders		
	N	Mean ± SD, median (quartile range), or *n* (%)	*N*	Mean ± SD, median (quartile range), or *n* (%)	*P* for difference or trend
Age, years	831	67.69 ± 4.16	235	68.71 ± 4.27	0.001
Male	831	430 (51.7)	235	117 (49.8)	0.596
Education	831		235		0.110
University degree	831	145 (17.4)	235	26 (11.1)	
Professional	831	239 (28.8)	235	68 (28.9)	
Secondary school	831	442 (53.2)	235	139 (59.1)	
Primary school	831	5 (0.6)	235	2 (0.9)	
SIMD rank	831		235		<0.001
1st quintile	831	99 (11.9)	235	28 (11.9)	
2nd quintile	831	143 (17.2)	235	65 (27.7)	
3rd quintile	831	143 (17.2)	235	45 (19.1)	
4th quintile	831	146 (17.6)	235	48 (20.4)	
5th quintile	831	300 (36.1)	235	49 (20.9)	
HADS anxiety	831	5 (3–8)	234	6 (4–9)	<0.001
HADS depression	831	3 (1–5)	234	4 (2–6)	0.002
Duration of diabetes, years	824	6 (3–11)	229	7 (3–12)	0.196
Current treatment	830		235		0.281
Insulin ± tablets	830	139 (16.7)	235	47 (20.0)	
Tablets alone	830	526 (63.4)	235	153 (64.8)	
Diet alone	830	165 (19.9)	235	35 (14.9)	
HbA_{1c}, %	804	7.39 ± 1.13	224	7.41 ± 1.08	0.872
HbA_{1c}, mmol/mol	804	57 ± 12.4	224	57 ± 11.8	0.872
Plasma glucose, mmol/L	821	7.54 ± 2.09	228	7.66 ± 2.13	0.425
Systolic BP, mmHg	829	132.51 ± 15.87	235	136.09 ± 18.09	0.006
Diastolic BP, mmHg	829	68.95 ± 8.87	235	69.44 ± 9.50	0.457
BMI, kg/m^2	831	31.29 ± 5.59	234	31.93 ± 6.03	0.130
WHR	828	0.97 ± 0.08	233	0.97 ± 0.08	0.966
Total cholesterol, mmol/L	826	4.34 ± 0.90	231	4.23 ± 0.91	0.100
Current smoker	831	108 (13.0)	235	45 (19.1)	0.018
Former smoker	831	390 (46.9)	235	109 (46.4)	0.882
Never smoked	831	333 (40.1)	235	81 (34.5)	0.120
cIMT, mm	775	1.00 ± 0.17	142	0.99 ± 0.17	0.408
ABI	824	0.99 ± 0.20	235	0.95 ± 0.23	0.009
NT-proBNP, pg/mL	820	71 (35–158)	230	102 (48–216)	0.004
MI	831	111 (13.4)	235	39 (16.6)	0.207
Angina	831	222 (26.7)	235	76 (32.3)	0.090
Stroke	831	44 (5.3)	235	18 (7.7)	0.171
TIA	831	27 (3.2)	235	4 (1.7)	0.213
PAD	831	53 (6.4)	235	12 (5.1)	0.472
Any CVD	831	293 (35.3)	235	100 (42.2)	0.041
MMSE	830	29 (28–30)	233	28 (27–29)	<0.001
MMSE <24	830	13 (1.6)	234	17 (7.3)	<0.001
Dementia	831	4 (0.5)	235	15 (6.4)	<0.001
MHVS	820	31.45 ± 5.07	229	29.07 ± 5.38	<0.001
LM	830	25.82 ± 7.97	232	22.94 ± 8.54	<0.001
Faces	829	66.35 ± 7.66	232	63.95 ± 8.40	<0.001
MR	830	13.35 ± 5.23	233	10.92 ± 5.02	<0.001
DSC	830	50.37 ± 14.37	231	44.72 ± 15.52	<0.001
TMT-B, sec	830	101 (79–132)	232	124 (94–164)	<0.001
Letter-Number Sequencing	829	9.91 ± 2.67	232	8.78 ± 2.95	<0.001
BVFS	828	37.77 ± 12.53	232	33.93 ± 13.47	<0.001
g	829	0.13 ± 0.93	231	−0.44 ± 1.11	<0.001

Attenders (n = 831) vs. nonattenders (n = 235). Data for attenders include three patients who attended follow-up but did not provide cognitive data. At baseline, 1,021 of 1,066 participants completed all cognitive tests. Missing data were imputed for 39 participants. BP, blood pressure; HADS, Hospital Anxiety and Depression Scale; PAD, peripheral arterial disease; SIMD, Scottish Index of Multiple Deprivation; WHR, waist-to-hip ratio.

for stroke and angina). History of TIA was associated with lower mean ABI (P = 0.009). Mean NT-proBNP ($P < 0.001$) and cIMT (P = 0.012) were increased in participants with MI.

Of all subjects attending follow-up (n = 831), three were not cognitively tested because of refusal or physical disability. Performance on each cognitive test correlated significantly with performance on all other cognitive tests at baseline (r = 0.19–0.63; all $P < 0.001$) and at follow-up (r = 0.26–0.65; all $P < 0.001$). Individual cognitive test scores and g correlated strongly between baseline and follow-up (Table 2). Mean scores declined slightly but significantly ($P < 0.001$) for all tests except Faces and LM, in which scores improved ($P < 0.001$), and for DSC (P = 0.150) (Table 2). Effect sizes and significance levels were similar when analyses were repeated using original rather than imputed data (data not shown).

At Bonferroni-corrected P = 0.006, follow-up g was found to be significantly lower in participants with a preexisting cardiovascular event at baseline (β = −0.18; $P < 0.001$); for individual event categories, findings were statistically significant for angina (β = −0.11; P = 0.002) and stroke (β = −0.16; $P < 0.001$). Lower follow-up g also appeared to be associated with measures of subclinical macrovascular disease, although only the association with higher NT-proBNP reached statistical significance at the Bonferroni-corrected level (β = −0.10; P = 0.005) (Table 3).

In multivariate analyses adjusting for baseline cognitive test scores or for MHVS (and for vascular risk factors), the only cardiovascular event category that remained significantly associated with lower g at follow-up was stroke (Table 3), suggesting that people with stroke experienced steeper cognitive decline compared with stroke-free individuals. For estimated lifetime cognitive change, the association was statistically significant at the Bonferroni-adjusted level of significance (β = −0.12; $P < 0.001$) and persisted after adjustment for cIMT (β = −0.10; P = 0.001). In terms of individual test scores, stroke appeared to affect predominantly processing speed (Supplementary Tables).

All markers of subclinical macrovascular disease were significantly associated with 4-year decline in g, although the association with ABI, which appeared to be driven by processing speed (TMT-B: β = −0.08; P = 0.001; Supplementary Tables), only lost statistical significance at the Bonferroni-adjusted level when vascular risk factors were controlled for (P = 0.009) (Table 3).

Further analyses of the individual cognitive tests revealed statistically significant associations of NT-proBNP with 4-year decline in verbal fluency at Bonferroni-corrected level (BVFT: β = −0.07; P = 0.001) (Supplementary Tables). Associations between increased cIMT and 4-year decline in processing speed (DSC: β = −0.08; P = 0.001) and reasoning (MR: β = −0.08; P = 0.004) appeared to be the main contributors to the association with decline in g. All associations of cIMT and NT-proBNP with g and individual cognitive tests (except between cIMT and MR; P = 0.007) remained statistically significant ($P < 0.006$) after adjustment for vascular risk factors (Table 3 and Supplementary Tables). In post hoc analyses, the association of cIMT with 4-year decline in g survived the additional adjustment for MHVS (β = −0.13; P = 0.001) as well as addition of stroke, ABI, and NT-proBNP into the model (β = −0.12; P = 0.002). The association of NT-proBNP with decline in g lost statistical significance when MHVS (P = 0.008) or when stroke, ABI, and cIMT were controlled for (P = 0.055).

A similar pattern of associations was found for the subclinical vascular markers and MHVS-adjusted cognitive test performance to signify estimated lifetime cognitive change, although overall the associations were weaker, such that only the association with cIMT reached the Bonferroni-adjusted level of statistical significance (β = −0.09; P = 0.005). The finding also became less significant when stroke was additionally controlled for (P = 0.011).

Only four subjects attending the year 4 follow-up had a diagnosis of dementia. Effect sizes and P values changed only marginally when analyses were repeated with exclusion of these cases (data not shown). Although there was a suggestion that the prevalence of vascular events was higher in all subjects with dementia, including TIA (P = 0.046) and MI (P = 0.027), this group of subjects (n = 19) was too small for further subgroup analysis. Twenty-three participants experienced conversion from normal functioning to MCI during follow-up. One (4.3%) had a history of TIA, three had stroke or peripheral arterial disease (13.0%, respectively), six (26.9%) had MI, 10 (43.5%) had angina, and 14 (60.9%) had any CVD. Age- and sex-adjusted analyses revealed increased prevalence of any CVD (P = 0.039) and significantly higher mean NT-proBNP levels (geometric mean: 131.1 pg/mL and 95% CI 81.1–211.7 vs. 74.07 pg/mL and 95% CI 68.4–80.3; P = 0.021) in the "conversion to MCI" group compared with the remaining population. None of the remaining macrovascular predictors was related to conversion to MCI (all $P > 0.05$).

Table 2—*Baseline and follow-up cognitive test performance of attenders*

	Correlation coefficient*	Baseline mean ± SD or median (IQR)	Follow-up mean ± SD or median (IQR)	*P* for difference†
MHVS	0.89	31.53 ± 5.02	30.70 ± 4.95	<0.001
g	0.84	0.12 ± 0.93	0.00 ± 1.00	<0.001
BVFT	0.81	37.83 ± 12.53	36.83 ± 12.75	<0.001
DSC	0.74	50.52 ± 14.30	50.01 ± 14.12	0.150
TMT-B, sec	0.66	104 (81–139)	107 (83–144)	<0.001
MR	0.67	13.36 ± 5.24	11.55 ± 5.22	<0.001
LNS	0.54	9.93 ± 2.66	8.86 ± 2.89	<0.001
LM	0.63	25.85 ± 7.97	27.27 ± 8.26	<0.001
Faces	0.61	66.41 ± 7.63	69.25 ± 8.38	<0.001
MMSE	0.51	29 (28–30)	29 (28–30)	0.011

N = 810–825. IQR, interquartile range; LNS, Letter-Number Sequencing. *Two-tailed Pearson correlations of baseline and follow-up cognitive scores (all $P < 0.001$). Data for the seven cognitive tests and g include imputed values. At baseline, missing data were imputed for 39 participants. At follow-up, missing data were imputed for 65 participants. †Between baseline and follow-up mean scores.

CONCLUSIONS—In this cohort of initially cognitively healthy older people with type 2 diabetes, markers of subclinical macrovascular disease, including higher circulating levels of the natriuretic peptide NT-proBNP and increased cIMT

Table 3—*Macrovascular disease and generalized cognitive ability* (g)

		4-year cognitive change		Estimated lifetime cognitive change	
	Age and sex-adjusted β	β adjusted for age, sex, and baseline score	Fully adjusted β	β adjusted for age, sex, and MHVS	Fully adjusted β
Vascular events					
Any vascular event (*n* = 393)	−0.18 (<0.001)	−0.09 (0.008)	−0.09 (0.014)	−0.06 (0.032)	−0.07 (0.023)
Stroke (*n* = 62)	−0.16 (<0.001)	−0.07 (0.036)	−0.07 (0.037)	−0.12 (<0.001)	−0.12 (<0.001)
TIA (*n* = 31)	−0.04 (0.239)	−0.05 (0.178)	−0.05 (0.177)	−0.02 (0.590)	−0.02 (0.580)
MI (*n* = 150)	−0.09 (0.014)	−0.04 (0.293)	−0.04 (0.278)	−0.05 (0.112)	−0.05 (0.096)
Angina (*n* = 298)	−0.11 (0.002)	−0.04 (0.237)	−0.04 (0.294)	−0.01 (0.752)	−0.01 (0.626)
Peripheral arterial disease (*n* = 65)	−0.06 (0.099)	−0.02 (0.567)	−0.01 (0.703)	−0.03 (0.266)	−0.03 (0.287)
Vascular markers					
NT-proBNP	−0.10 (0.005)	−0.12 (0.001)	−0.10 (0.005)	−0.07 (0.013)	−0.07 (0.017)
ABI	0.10 (0.007)	0.12 (0.001)	0.10 (0.009)	0.05 (0.062)	0.05 (0.130)
cIMT	−0.09 (0.015)	−0.15 (<0.001)	−0.12 (0.001)	−0.10 (0.002)	−0.09 (0.005)

Outcome variable is follow-up *g*. β values are standardized regression coefficients. Values in parentheses are *P* values. Cognitive data include imputed values. *N* = 755–823. Final models additionally adjusted for baseline vascular risk factors (total cholesterol, brachial blood pressure, cigarette smoking).

as well as a history of stroke, were associated with cognitive decline.

Stroke is a well-established risk factor associated with cognitive dysfunction and impairment (28), and also may contribute to accelerated cognitive decline (29), although the relationship in exclusively diabetic populations has not been extensively investigated. In the ET2DS, stroke was associated with steeper cognitive decline between peak premorbid ability, which was estimated by vocabulary, and late-life ability. Contrary to previous investigations in older adults with type 2 diabetes (30,31), stroke also appeared to be associated with rate of actual late-life cognitive decline over the 4-year follow-up, although these associations were weaker and less statistically significant, suggesting that cognitive function deteriorates from prestroke to immediate poststroke levels, with preexisting stroke having relatively lower impact on subsequent cognitive decline.

The associations of cognitive decline with subclinical measures of macrovascular disease, including higher levels of NT-proBNP, increased thickness of the cIMT, and lower ankle brachial pressure (a further measure of systemic atherosclerotic disease as well as of lower limb peripheral arterial disease) (32), appeared slightly stronger for 4-year cognitive decline compared with associations of these vascular markers with estimated lifetime cognitive decline. This pattern of results is of interest because, in terms of identifying elderly subjects at risk for subsequent cognitive decline, information on a patient's future decline may be more valuable compared with information that incorporates past decline. Higher natriuretic peptide levels as a marker of cardiac stress previously have been associated with low level of cognitive function (33) and risk of future dementia (34). However, to our knowledge, this was the first prospective investigation of NT-proBNP and cognitive decline in a cognitively healthy population with type 2 diabetes. In addition to associations with continuously measured decline in ability, higher baseline peptide levels also predicted increased risk of conversion from normal cognitive functioning to suspected MCI. Increased cIMT has been shown to be a marker of coexistent CVD, including cerebrovascular disease (35), and may indicate systemic atherosclerosis as well as more localized disease. Evidence for associations with cognitive decline is mixed (36,37), however, and although our finding of accelerated 4-year cognitive decline in individuals with higher cIMT is consistent with one previous study performed in a nondiabetic population (36), investigations of patients with type 2 diabetes are lacking. For cIMT, but not for NT-proBNP, associations in the current study were unrelated to potential effects of premorbid ability in late-life cognitive decline and risk of late-life subclinical vascular damage.

Contrary to previous investigations performed in predominantly nondiabetic populations (38,39), we found an association between low ABI and steeper late-life cognitive decline. Individuals exposed to lifestyle-associated vascular risk, such as cigarette smoking, hypertension, and hypercholesterolemia, may develop subclinical macrovascular disease, evident in lower ABI, and also may experience accelerated late-life cognitive decline. However, the association of ABI with cognitive decline was only partially dependent on conventional cardiovascular risk factors.

It is also possible that the ABI may function as a vascular risk marker in a slightly different manner in diabetic populations because of effects of stiffened arteries on the measurement of ankle pressures (12), and this could contribute to any disparity between findings for ABI and cognitive decline in diabetic compared with nondiabetic populations.

The assessment of links between vascular disease and cognition is notoriously difficult because of the interrelationships between different vascular measures and the problems associated with measuring or estimating change in cognitive abilities over appropriate time periods in relation to the development of vascular disease markers. This somewhat explains inconsistencies in the current literature on the role of large and small vessel vascular disease in the etiology of cognitive decline. Compared with some previous studies, the relatively large size, population-based approach, and prospective design are strengths of the current study. The use of an exclusively diabetic older population and the measurement of a large number of different but commonly used markers of subclinical and clinical macrovascular disease provides a unique contribution to the existing literature that has neglected investigations in diabetes patients despite increased prevalence of macrovascular disease and cognitive impairment (5,8,11). The general ability factor is a universal empirical finding, a robust measure of overall cognitive functioning, and also the locus of much of the age effects on cognition (1). A conservative Bonferroni correction was applied to the cut-point for statistical significance; results were discussed both in terms of the corrected and the traditional cut-point ($P < 0.05$) with the aim of offering an overall interpretation of findings with minimal risk of statistical error. Adjustment for a vocabulary-based cognitive test enabled estimation of long-term change in ability, although the MHVS only served as an estimate of peak premorbid ability and caution is warranted in the interpretation of this outcome. Analyses of actual longitudinal change in cognitive test scores were presumably subject to some error. Practice effects and selective attrition, which favor participants with slower rates of decline (40), lead to underestimation of actual cognitive decline in the population. Individuals with higher ability at baseline were more likely to attend follow-up and overall 4-year cognitive decline for attenders was small. Although we set out to recruit a cognitively healthy population, subsequent consideration of information from a wide variety of sources revealed that a very small number of subjects were likely to have been experiencing dementia at baseline. However, exclusion of dementia cases from the analyses only marginally changed the results reported here.

Our results provide additional evidence that the impact of vascular disease on cognition may not be restricted to localized cerebral small vessel disease or altered blood flow and ischemic damage as a consequence of stroke, and that cognitive decline could reflect systemic atherosclerotic changes. NT-proBNP and cIMT both appear to function as biomarkers of risk of late-life cognitive decline, despite their strong associations with subclinical macrovascular disease in different areas of the vasculature (left ventricle and carotid artery, respectively) and may offer valuable information beyond traditional vascular risk factors. Given that stroke was only weakly associated with 4-year cognitive decline, and that none of the remaining measures of symptomatic macrovascular disease were related to this outcome, determination of subclinical macrovascular disease using continuous measures may be preferable over symptomatic categorically assessed macrovascular disease for the early identification of individuals at risk for late-life cognitive decline. However, it must be recognized that the categorical nature of "events" data compared with the continuous distribution of the subclinical measures is likely to have influenced the relative levels of statistical significance for these variables. Ascertainment of subclinical macrovascular disease may aid the identification of not only individuals at risk for future symptomatic macrovascular disease (and its cognitive consequences) but also those at risk for late-life cognitive decline in absence of symptomatic disease. With the proximity of the carotid artery to the brain, cIMT could be hypothesized to represent the most accurate noninvasive marker of asymptomatic cerebral vascular damage. Certainly, its potential as a vascular risk marker seems to exceed that of simply a risk factor for stroke.

In conclusion, we have found confirmatory evidence of a link between stroke and cognitive decline in people with type 2 diabetes but, more importantly, we have identified associations between a number of different measures of subclinical macrovascular disease and late-life cognitive change. Further studies are warranted to determine whether these markers could be useful clinical measures as risk predictors for cognitive impairment and subsequent targeting of interventions aimed at reducing cognitive decline.

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I.F. performed the statistical analysis and drafted the manuscript. M.K., C.M.R., J.R.M., R.M.W., L.D.N., S.M., N.S., P.W., R.M.R., T.C.R., M.W.J.S., and J.F.P. were involved in the interpretation of findings and preparation of the final manuscript, including commenting on the final draft. I.J.D. supervised statistical analysis and was involved in the interpretation of findings and preparation of the final manuscript, including commenting on the final draft. M.W.J.S. conceived and designed the ET2DS, oversaw the acquisition and analysis of data, and was involved in the interpretation of findings and preparation of the final manuscript, including commenting on the final draft. J.F.P. supervised statistical analysis, conceived and designed the ET2DS, and oversaw the acquisition and analysis of data. J.F.P. and I.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Severe Hypoglycemia and Cognitive Decline in Older People With Type 2 Diabetes: The Edinburgh Type 2 Diabetes Study

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OBJECTIVE

People with type 2 diabetes are at increased risk of age-related cognitive decline and dementia. Hypoglycemia is a candidate risk factor, but the direction of association between episodes of severe hypoglycemia and cognitive decline in type 2 diabetes remains uncertain.

RESEARCH DESIGN AND METHODS

In the Edinburgh Type 2 Diabetes Study, cognitive function was assessed in 831 adults with type 2 diabetes (aged 60–75 years) at baseline and after 4 years. Scores on seven neuropsychological tests were combined into a standardized general ability factor *g*. Self-reported history of severe hypoglycemia at baseline (history of hypoglycemia) and at follow-up (incident hypoglycemia) was recorded.

RESULTS

A history of hypoglycemia was reported by 9.3% of subjects, and 10.2% reported incident hypoglycemia. Incident hypoglycemia was associated with poorer cognitive ability at baseline (age- and sex-adjusted odds ratio for lowest tertile of *g* 2.04 [95% CI 1.25–3.31], *P* = 0.004). Both history of hypoglycemia and incident hypoglycemia were also associated with greater cognitive decline during follow-up (mean follow-up *g* adjusted for age, sex, and baseline *g* −0.25 vs. 0.03 [*P* = 0.02] and −0.28 vs. 0.04 [*P* = 0.01], respectively), including after addition of vascular risk factors and cardiovascular and microvascular disease to the models (−0.23 vs. 0.03 [*P* = 0.04] and −0.21 vs. 0.05 [*P* = 0.03], respectively).

CONCLUSIONS

The relationship between cognitive impairment and hypoglycemia appeared complex, with severe hypoglycemia associated with both poorer initial cognitive ability and accelerated cognitive decline.

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Type 2 diabetes is associated with an increased risk of cognitive impairment, age-related cognitive decline, and dementia (1). Given the increasing numbers of elderly people with type 2 diabetes in the general population, the identification of potentially modifiable risk factors and the prevention of cognitive decline during older age in this group are of major importance to public health. Although the

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PATHOPHYSIOLOGY/COMPLICATIONS

mechanisms underlying progressive cognitive impairment are likely to be multifactorial (2), cerebral insults associated with diabetes-associated episodes of severe hypoglycemia (those in which a patient requires external assistance to aid recovery) are possible contributors. In studies of individuals with type 1 diabetes, in whom relatively frequent episodes of severe hypoglycemia are well-recognized, severe hypoglycemia has been associated with lower cognitive ability and implicated in provoking cognitive decline in children (3) but not in adults (4). However, severe hypoglycemia is also relatively common in adults with insulin-treated type 2 diabetes and to a lesser extent in individuals treated with sulfonylureas (5). Because type 2 diabetes is predominant in the older population, the investigation of the relationship between hypoglycemia and age-related cognitive decline in this group, which has received limited attention, is particularly pressing.

Retrospective analyses of hospital records have suggested that exposure to one or more episodes of severe hypoglycemia is associated with an increase in the subsequent risk of dementia in people with type 2 diabetes (6–9). These findings may be inflated by the higher incidence of hypoglycemia in hospital inpatients than in patients in community settings (10) but are supported by observations of cross-sectional links between a history of severe hypoglycemia and impaired cognitive function short of frank dementia (11,12). However, the extent to which these associations are explained by hypoglycemia occurring as a consequence of poor cognitive ability leading to suboptimal glycemic control (13) as opposed to hypoglycemia preceding and possibly causing decrements in cognitive ability is unknown. The very few prospective analyses performed to date (which might help to resolve this question) have not implicated severe hypoglycemia as a risk factor for negative cognitive outcome (14–16). However, these studies either have neglected relatively mild cognitive decline (14) or have been part of randomized controlled trials in which the nature of the intervention itself potentially affected the relationship between severe hypoglycemia and cognitive ability (15,16). The principal aim of the current study was to determine the association of both prevalent and incident severe hypoglycemia with cognitive decline measured prospectively through a range of age-sensitive cognitive tests in a representative sample of older adults with type 2 diabetes living independently in the general population and participating in a well-established observational epidemiological study (the Edinburgh Type 2 Diabetes Study [ET2DS]).

RESEARCH DESIGN AND METHODS

Study Population

Recruitment and examination procedures for the prospective ET2DS have been reported previously (17). In brief, in 2006/2007, a sample of 1,066 men and women with type 2 diabetes (aged 60–75 years), largely representative of all individuals invited at random from a population-based diabetes register (18), attended a baseline clinic. In 2010/2011, 831 participants (attenders) returned for a 4-year follow-up; nonattenders were followed up through postal questionnaires, linkage to hospital records, death certificate data, and review of hospital notes. All participants gave written informed consent.

Clinical Examination

At the baseline clinic and year-4 follow-up, HDL cholesterol, total cholesterol, and plasma HbA_{1c} concentrations were measured in fasting blood samples; systolic and diastolic brachial blood pressures were measured; and smoking history (current, never, and former) was self-reported. Diabetic retinopathy was assessed at baseline as absent, mild, or moderate/severe on the basis of seven-field retinal photographs. History of myocardial infarction (MI), angina, stroke, and transient ischemic attack (TIA) was determined at baseline through self-report of a physician diagnosis, World Health Organization chest pain questionnaire, 12-lead electrocardiogram, and linkage to hospital discharge records, as detailed previously (18). The same sources of data and criteria were used to ascertain incident MI, angina, stroke, and TIA events between baseline and year 4. Scores on the self-administered depression subscale of the Hospital Anxiety and Depression Scale (HADS-D) (19) (score range 0–21) measured symptoms of depression at baseline and at year 4. Scores $<$24 of 30 on the Mini-Mental State Examination (20) were used together with additional criteria (21) to identify participants with dementia by year 4 follow-up.

Measurement of Hypoglycemia

Reporting of severe hypoglycemia in the ET2DS is summarized in Fig. 1. At baseline, a questionnaire determined participants' history of severe hypoglycemia (history of hypoglycemia), which was defined as an episode of hypoglycemia requiring the assistance of another person to effect recovery. Participants were asked about the number of episodes they had experienced over their lifetime and within the past year. A similar questionnaire at the year 4 examination determined severe hypoglycemia since baseline (incident hypoglycemia), including the number of episodes experienced in total, and in the year preceding the examination. Data from participants who expressed uncertainty were not used.

Additionally, 898 consenting participants were enrolled in a detailed 6-month survey of severe hypoglycemia, commencing ~1 year after baseline. Once every 2 months for a total of 6 months, participants returned self-completed questionnaires based on the Edinburgh Hypoglycemia scale (22) that comprised items on symptoms, date and time of any hypoglycemic episode, loss of consciousness, help from another person, treatment, and blood glucose values, if measured. For those who reported severe hypoglycemia or who failed to return a questionnaire, data were obtained and verified by telephone.

Cognitive Assessment at Baseline and Year 4

With the aim to minimize effects of measurement error on the baseline and year 4 cognitive test data (a prerequisite for the validity of analyses of cognitive

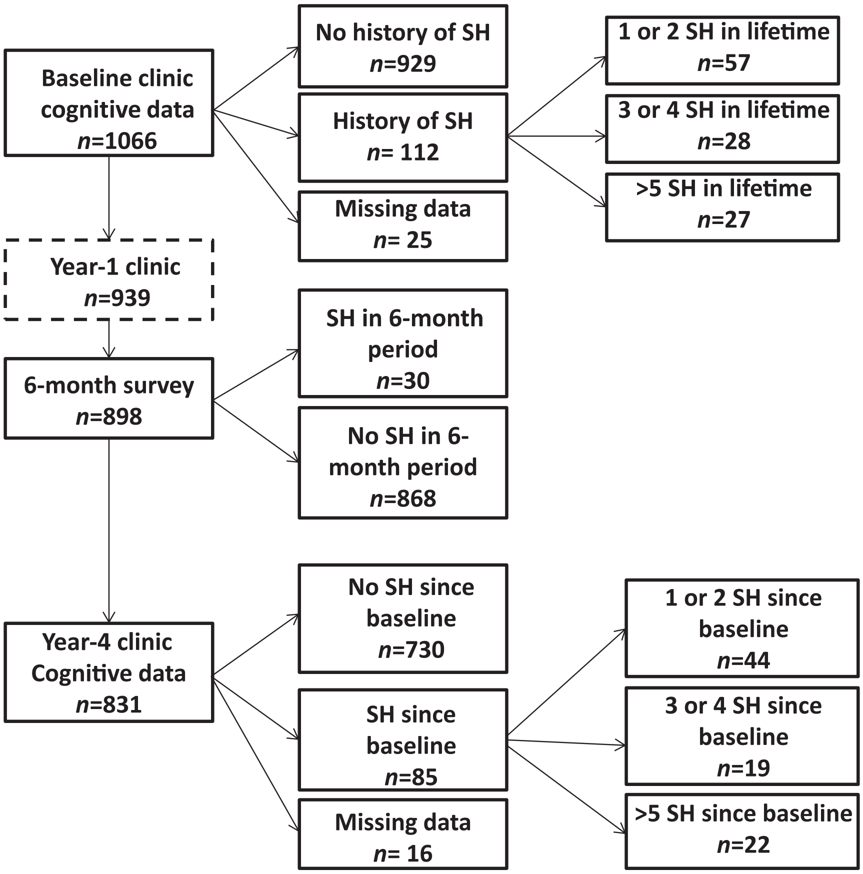

Figure 1—Reporting of severe hypoglycemia (SH) in the ET2DS.

change), conditions for cognitive testing were kept the same at the two time points as much as possible. At both times, the absence of current hypoglycemia was confirmed by measuring blood glucose before commencing each testing session, which was only undertaken if values were ≥4.0 mmol/L. Participants were also reminded by letter to bring their glasses or hearing aids as required and were prompted to use these at the clinic. Data from participants with severe sensory impairment were not used. The Logical Memory (LM) and Faces subtests of the Wechsler Memory Scale—Third Edition (U.K.) assessed verbal and nonverbal memory, respectively (23). Executive function was measured with the Borkowski Verbal Fluency Test (BVFT) and the Trail Making Test B (TMT-B). The Digit Symbol—Coding (DSC), Letter-Number Sequencing (LNS), and Matrix Reasoning (MR) subtests of the Wechsler Adult Intelligence Scale—Third Edition (U.K.) assessed speed of processing, working memory, and nonverbal reasoning, respectively (24). Peak premorbid ability was estimated by the junior and senior Mill Hill Vocabulary Scale (MHVS) (25), a valid measure of crystallized intelligence relatively immune to age-related decline (26).

Statistical Analysis

TMT-B, HADS-D, and duration of diabetes were transformed to their natural logarithms because of skewed distributions. Data were missing on the seven age-sensitive cognitive tests (LM, Faces, MR, DSC, TMT-B, LNS, BVFT) for between 0.6% (BVFT) and 1.7% (LNS) of participants at baseline and for between 0.8% (MR) and 4.7% (LNS) at year 4 follow-up. Multiple imputation accounting for age and sex was carried out for participants with missing data on one, two, or three cognitive tests. Different cognitive tests tending to load on a single factor of global cognitive ability, which has been termed *g* (27), is commonly observed. With the aim to extract *g*, components with eigenvalues >1 were extracted from a principal components analysis of scores on the seven cognitive tests after their imputation. All seven cognitive tests loaded on a single component with an eigenvalue >1; inspection of the scree plot also suggested a strong general cognitive ability factor. The use of the standardized factor *g* derived from this method is advantageous because in contrast to scores on individual cognitive tests, it partly offsets test-specific measurement error. In the ET2DS, *g* has been found to capture participants' overall performance at baseline and year 4 follow-up, as described previously (21).

All analyses were adjusted for age and sex. Initially, ANCOVA was used to compare mean baseline *g* between individuals with incident hypoglycemia and the remaining sample. This method does not entirely eliminate the influence from potential confounders to allow their dismissal, especially in observational studies, but reduces covariate-associated noise in the data (28); therefore, it was used to address the issue of confounding in the current study. Additionally, odds ratios (ORs) for incident hypoglycemia were calculated in participants with low baseline *g* (i.e., who scored in the lowest tertile of *g*) in logistic regression models. Analyses were repeated with restriction to incidence of first-ever incident hypoglycemia.

Further logistic regression analyses ascertained the OR for reduced follow-up *g* and accelerated decline in *g* (both scoring in the lowest tertiles of distribution) according to history of previous hypoglycemia at baseline and of subsequent incident hypoglycemia between baseline and year 4. Additionally, ANCOVA was used to determine hypoglycemia associations with follow-up cognitive test performance and cognitive change. Cognitive change between baseline and year 4 was represented by inclusion of baseline cognitive test scores in the models of follow-up scores. This method was chosen over the alternative of raw change scores because its parameter estimates are more straightforward and no assumptions are made regarding the group difference in cognitive function at baseline (29).

Cognitive change between estimated peak premorbid ability and year 4 ability was represented by inclusion of baseline MHVS in models of year 4 cognitive test scores. For all outcomes, vascular risk factors (HDL and total cholesterol, systolic and diastolic blood pressure, smoking), HbA_{1c}, cerebrovascular disease (stroke, TIA), coronary heart disease (angina, MI), and diabetic retinopathy as a measure of microvascular disease (vascular covariates) were then added to the models. Treatment modality, duration of diabetes, and HADS-D were also included in analyses performed post hoc and presented in the text. Baseline covariates were used for analyses of a baseline history of hypoglycemia, and year 4 covariates were used in analyses of incident hypoglycemia. An exception to this was smoking status, which was found to be relatively stable over the course of the study, and retinopathy, which was measured only at baseline.

Analyses were performed with SPSS version 19.0 (IBM Corporation, Armonk, NY) statistical software.

RESULTS

Characteristics of Follow-up Study Population

Characteristics of attenders of the year 4 follow-up are presented in Table 1. Attenders were largely similar to nonattenders in terms of baseline clinical characteristics but had higher baseline cognitive function (21). In addition, nonattendance was significantly associated with a baseline history of hypoglycemia (31.3 and 20.5% of participants with and without a history of hypoglycemia at baseline, respectively, were nonattenders; P = 0.01).

History of Hypoglycemia

In the total study population attending follow-up (n = 831), 77 (9.3%) participants reported a history of hypoglycemia at baseline and 85 (10.2%) experienced incident hypoglycemia. Of the latter, 27 (31.8%) also had a baseline history of hypoglycemia ($P < 0.001$ for risk of recurrent severe hypoglycemia). Of 898 participants enrolled in the 6-month hypoglycemia survey, 30 reported a total of 45 episodes of severe hypoglycemia. For 18 of the 45 episodes in which blood glucose readings were self-reported, all but 1 of 14 measurements made before treatment were <3.0 mmol/L, suggesting that participants were reasonably accurate at identifying hypoglycemia. Of the 30 participants reporting hypoglycemia in the 6-month survey, 25 recovered after treatment with a glucose drink or ingesting food, 1 required injection with glucagon, and 3 required intravenous glucose (information was missing for 1 participant). Twenty-three of the 30 participants attended the year 4 follow-up; most (74%) reported incident hypoglycemia in the 4 years since baseline, demonstrating a reasonable level of recall of hypoglycemia at follow-up.

Association of Baseline Cognitive Function With Incident Hypoglycemia

Baseline global cognitive function was lower in participants with incident hypoglycemia than in those remaining free of incident hypoglycemia (age- and sex-adjusted mean *g* −0.08 [95% CI −0.27 to 0.12] vs. 0.17 [0.10–0.24], P = 0.019). Participants scoring in the lowest tertile of *g* were at a twofold higher risk of experiencing incident hypoglycemia compared with higher-scoring participants (age- and sex adjusted OR 2.04 [95% CI 1.25–3.31], P = 0.004). Results were largely unchanged when restricted to participants who experienced their first-ever severe hypoglycemic episode during the follow-up period (age- and sex-adjusted OR 2.45 [1.37–4.39], P = 0.002; adjusted mean *g* −0.08 [−0.33 to 0.16] vs. 0.18 [0.12–0.25], P = 0.038). The presence of dementia in four participants was not significantly associated with incident hypoglycemia ($P > 0.05$). Because their exclusion did not alter P values and effect sizes (data not shown), results are reported for the entire sample.

Baseline History of Hypoglycemia, Cognitive Function, and Cognitive Change

Participants with a history of hypoglycemia had lower performance on MR, DSC, TMT-B, and *g* at year 4 (Table 2) and were marginally more likely to score in the lowest tertile of *g* (age- and sex-adjusted OR 1.65 [0.99–2.76], P = 0.055) compared with the remaining population. All associations persisted when MHVS (which estimates premorbid ability) was included in the model (all $P < 0.05$) (data not shown). All except DSC (P = 0.145) survived further addition of baseline vascular covariates, baseline HADS-D, duration of diabetes, and baseline treatment modality into the model (all $P < 0.05$) (data not shown).

A history of hypoglycemia was also associated with a steeper decline between baseline and year 4 on MR, TMT-B, and *g* (Table 2), although the OR for cognitive decline (lowest tertile of standardized residuals signifying a 4-year decline in *g*) was not statistically significant (1.36 [0.82–2.24], P = 0.230). Inclusion of baseline vascular covariates marginally attenuated the associations with MR and *g* (Table 2). For MR ($P <$ 0.05) but not *g* (P = 0.083), the association remained statistically significant when baseline HADS-D,

Table 1—Characteristics of year 4 attenders according to incident hypoglycemia reported at follow-up

	All attenders (maximum *n* = 831)	Incident hypoglycemia (maximum *n* = 85)	No incident hypoglycemia (maximum *n* = 730)	*P* value for difference or trend*
Age (years)	67.69 ± 4.16	66.69 ± 4.05	67.79 ± 4.17	0.022
Male sex	430 (51.7)	32 (37.6)	939 (53.8)	0.005
Duration of diabetes (years)	6.00 (3.00–11.00)	9.50 (5.25–15.00)	6.00 (300–10.00)	<0.001
Baseline treatment				<0.001
Insulin ± tablets	139 (16.7)	31 (36.5)	103 (14.1)	
Sulfonylureas ± other tablets	210 (25.3)	28 (32.9)	179 (24.6)	
Other tablets	316 (38.0)	19 (22.4)	292 (40.1)	
Diet alone	165 (19.9)	7 (8.2)	155 (21.3)	
Year 4 treatment				<0.001
Insulin ± tablets	178 (21.4)	40 (47.1)	133 (18.2)	
Sulfonylureas ± other tablets	255 (30.7)	25 (29.4)	228 (31.2)	
Other tablets	283 (34.1)	16 (18.8)	261 (35.8)	
Diet alone	115 (13.8)	4 (4.7)	108 (14.8)	
Plasma HbA_{1c} (%)	7.39 ± 1.13	7.86 ± 1.22	7.32 ± 1.06	<0.001
Plasma HbA_{1c} (mmol/mol)	57 ± 12.4	62 ± 13.3	56 ± 11.6	<0.001
Systolic BP (mmHg)	133 ± 16	130 ± 15	132 ± 16	0.086
Diastolic BP (mmHg)	69 ± 9	67 ± 6	69 ± 9	0.002
Vascular disease				
MI	111 (13.4)	12 (14.1)	98 (13.4)	0.860
Angina	222 (26.7)	22 (25.9)	197 (27.0)	0.828
Stroke	44 (5.3)	2 (2.4)	42 (5.8)	0.189
TIA	27 (3.2)	7 (8.2)	19 (2.6)	0.005
Retinopathy	266 (32.0)	33 (39.3)	227 (31.5)	0.150
Total cholesterol (mmol/L)	4.34 ± 0.90	4.34 ± 0.81	4.32 ± 0.90	0.890
HDL cholesterol (mmol/L)	1.29 ± 0.36	1.26 ± 0.35	1.29 ± 0.36	0.351
Smoking status				
Current smoker	108 (13.0)	18 (21.2)	88 (12.1)	0.018
Ex-smoker	390 (46.9)	37 (43.5)	349 (47.8)	0.455
Never smoked	333 (40.1)	30 (35.3)	293 (40.1)	0.388
HADS-D	3 (1–6)	5 (2–7)	3 (1–5)	<0.001
MMSE	28.47 ± 1.64	28.59 ± 1.48	28.51 ± 1.57	0.647
Dementia	4 (0.5)	0 (0.0)	2 (0.3)	0.629
MHVS	31.45 ± 5.07	30.80 ± 4.84	31.63 ± 5.07	0.151

Data are mean ± SD, median (interquartile range), or *n* (%). Data are from baseline unless otherwise indicated. BP, blood pressure; MMSE, Mini-Mental State Examination. *Comparing the incident hypoglycemia with the no incident hypoglycemia group.

baseline treatment, and disease duration were added to the model (data not shown).

Incident Hypoglycemia, Cognitive Function, and Cognitive Change

Incident hypoglycemia was associated with lower *g*, MR, LNS, TMT-B, DSC, and Faces performance at year 4 (Table 3). The risk of reduced cognitive function at follow-up (scoring in the lowest tertile *g*) was increased threefold for the incident hypoglycemia group (age- and sex-adjusted OR 2.97 [1.82–4.86], $P < 0.001$). All associations remained statistically significant when MHVS was added to the respective models, suggesting that participants with incident hypoglycemia experienced a steeper estimated lifetime decline between their peak premorbid and late-life ability than did the group free of incident hypoglycemia (Table 3). All associations except for DSC further survived the inclusion of year 4 vascular covariates (Table 3) and the inclusion of HADS-D at year 4, treatment at year 4, and disease duration in the respective models (all $P < 0.05$) (data not shown).

Participants with incident hypoglycemia also experienced a steeper decline between baseline and year 4 in MR, TMT-B, DSC, Faces, and *g* (Table 3). Those with the highest rate of decline (scoring in the lowest tertile of standardized residuals signifying a decline in *g*) were marginally more likely to have experienced incident hypoglycemia than the remaining population (age- and sex-adjusted OR 1.53 [0.95–2.47], $P = 0.084$). The associations with decline in MR, TMT-B, Faces, and *g* survived inclusion of year 4 vascular covariates (Table 3). The association with decline in Faces and MR further survived addition of HADS-D at year 4, treatment at year 4, and disease duration to the model (both $P < 0.05$); for TMT-B and *g*, the findings were just short of statistical significance ($P = 0.064$ and 0.072, respectively) (data not shown).

CONCLUSIONS

Lower cognitive ability at baseline was associated with a twofold higher incidence of severe hypoglycemia over 4 years. In addition, severe hypoglycemia was associated with a steeper decline in cognitive function. The latter was observed when cognitive change was

Table 2—Association of baseline history of hypoglycemia with cognitive function at year 4 follow-up and 4-year cognitive decline

	No baseline history (n = 739)	Baseline history (n = 77)	P value for group difference	Effect size of group difference (partial η^2)
Model 1: age, sex				
MR	11.76 (11.39 to 12.13)	10.14 (8.97 to 11.31)	0.009	0.008
LNS	8.96 (8.75 to 9.16)	8.33 (7.67 to 8.99)	0.076	0.004
BVFT	37.19 (36.27 to 38.11)	34.63 (31.72 to 37.54)	0.100	0.003
DSC	50.51 (49.53 to 51.49)	46.31 (43.19 to 49.44)	0.012	0.012
ln(TMT-B)	109.84 (106.70 to 113.07)	129.02 (117.68 to 141.60)	0.001	0.013
Faces	69.37 (68.78 to 69.96)	68.70 (66.85 to 70.56)	0.500	0.001
LM	27.28 (26.69 to 27.84)	27.71 (25.86 to 29.56)	0.661	<0.001
g	0.04 (−0.03 to 0.11)	−0.26 (−0.49 to −0.04)	0.009	0.008
Model 2: age, sex, baseline score				
MR	11.75 (11.47 to 12.03)	10.22 (9.34 to 11.11)	0.001	0.013
LNS	8.92 (8.74 to 9.09)	866 (8.10 to 9.22)	0.394	0.001
BVFT	37.04 (36.50 to 37.59)	36.17 (34.45 to 37.89)	0.340	0.001
DSC	50.31 (49.63 to 50.99)	48.31 (46.14 to 50.49)	0.086	0.004
ln(TMT-B)	110.72 (108.20 to 113.18)	119.94 (111.72 to 128.90)	0.036	0.005
Faces	69.40 (68.92 to 69.87)	68.49 (66.98 to 70.00)	0.260	0.002
LM	27.31 (26.85 to 27.77)	27.34 (25.89 to 28.80)	0.966	<0.001
g	0.03 (−0.04 to 0.10)	−0.25 (−0.48 to −0.02)	0.020	0.007
Model 3*				
MR	11.82 (11.53 to 12.11)	10.35 (9.41 to 11.29)	0.004	0.011
LNS	8.95 (8.77 to 9.13)	8.70 (8.12 to 9.29)	0.423	0.001
BVFT	37.33 (36.77 to 37.89)	36.47 (34.65 to 38.30)	0.381	0.001
DSC	50.38 (49.79 to 51.08)	48.73 (46.46 to 51.01)	0.176	0.002
ln(TMT-B)	109.95 (107.45 to 112.51)	117.57 (109.07 to 126.72)	0.097	0.004
Faces	69.52 (69.03 to 70.01)	68.81 (67.21 to 70.41)	0.409	0.001
LM	27.45 (26.98 to 27.91)	27.93 (26.42 to 29.45)	0.549	<0.001
g	0.03 (−0.04 to 0.11)	−0.23 (−0.47 to 0.01)	0.040	0.006

Data are adjusted mean (95% CI). n = 808–811 for model 1; n = 807–810 for model 2; n = 768–770 for model 3. Means for ln(TMT-B) are geometric means. *Adjusted for age, sex, baseline score, and baseline data on HDL cholesterol, total cholesterol, systolic blood pressure, diastolic blood pressure, smoking, HbA_{1c}, stroke, TIA, angina, MI, and retinopathy.

measured subsequently to the hypoglycemic events and, even more strongly, when the two were occurring simultaneously during the 4-year follow-up period. Thus, the results suggest that the experience of severe hypoglycemia may be associated with lesser prior cognitive ability and is a risk factor for accelerated cognitive decline. In addition to global cognitive ability measured by g, associations with cognitive outcome were most consistently observed for processing speed, nonverbal memory, executive function, and reasoning.

Previous cross-sectional studies demonstrated a relationship between hypoglycemia and poorer cognitive function in people with type 2 diabetes (11,12,14). However, uncertainty remains about the reasons for this association and the direction of any possible causal relationship between hypoglycemia and cognitive decrements. Evidence supports that people with poorer cognitive ability may be more susceptible to hypoglycemia. Lower baseline scores on a screening instrument for dementia predicted 5-year incident severe hypoglycemia in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial (15). Clinically diagnosed dementia has been shown to predict a two- to threefold increased risk of hospital admission or emergency treatment for hypoglycemia in the Fremantle Diabetes Study (14) and in the Health, Aging and Body Composition Study (9). In the Memory in Diabetes study of the Action to Control Cardiovascular Risk in Diabetes (ACCORD-MIND) trial, lower baseline cognitive test scores also predicted an increased risk of a first-ever hypoglycemic episode over 3.5 years of follow-up (11). These findings are consistent with the current study in which baseline g predicted subsequent severe hypoglycemia. It is possible that people with lower or declining cognitive ability are less able to recognize hypoglycemia, to treat it appropriately when it occurs, or to prevent it through modification of diabetes therapy.

Less epidemiological evidence supports the hypothesis that hypoglycemia may have a direct or indirect effect on the brain, resulting in cognitive decrements. In type 1 diabetes, the balance of evidence suggests that hypoglycemia may not affect cognitive function in this way (30). Incidence of severe hypoglycemia over 18 years was not associated with concurrent cognitive decline in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications cohort (4), and in a smaller study, 10-year incidence of severe hypoglycemia failed to predict

Table 3—Association of incident hypoglycemia with cognitive function at year 4 follow-up and with 4-year and estimated lifetime cognitive decline

	No incident hypoglycemia (n = 730)	Incident hypoglycemia (n = 85)	P value for group difference	Effect size of group difference (partial η^2)
Cognitive ability at year 4				
Model 1: age, sex				
MR	11.82 (11.45 to 12.19)	10.02 (8.92 to 11.12)	0.003	0.011
LNS	9.02 (8.82 to 9.23)	8.00 (7.38 to 8.61)	0.002	0.012
BVFT	37.20 (36.27 to 38.12)	35.34 (32.60 to 38.09)	0.211	0.002
DSC	50.69 (49.70 to 51.67)	46.63 (43.69 to 49.57)	0.011	0.008
ln(TMT-B)	109.29 (106.16 to 112.51)	126.85 (116.28 to 138.52)	0.001	0.013
Faces	69.63 (69.05 to 70.22)	67.08 (65.33 to 68.83)	0.007	0.009
LM	27.42 (26.83 to 28.01)	26.77 (25.00 to 28.54)	0.493	0.001
g	0.07 (0.00 to 0.13)	−0.33 (−0.53 to −0.12)	<0.001	0.015
Four-year cognitive decline				
Model 2*				
MR	11.75 (11.47 to 12.04)	10.57 (9.72 to 11.41)	0.009	0.008
LNS	8.97 (8.79 to 9.14)	8.42 (7.89 to 8.94)	0.052	0.005
BVFT	37.15 (36.61 to 37.70)	35.77 (34.14 to 37.40)	0.116	0.003
DSC	50.51 (49.82 to 51.19)	48.21 (46.16 to 50.26)	0.038	0.005
ln(TMT-B)	109.95 (107.55 to 112.51)	119.82 (112.06 to 128.25)	0.019	0.007
Faces	69.59 (69.11 to 70.07)	67.53 (66.11 to 68.96)	0.008	0.009
LM	27.36 (26.90 to 27.83)	27.27 (25.87 to 28.66)	0.900	<0.001
g	0.04 (−0.03 to 0.12)	−0.28 (−0.49 to −0.06)	0.006	0.009
Model 3†				
MR	11.78 (11.49 to 12.07)	10.65 (9.77 to 11.53)	0.017	0.007
LNS	8.98 (8.80 to 9.16)	8.54 (7.99 to 9.09)	0.140	0.003
BVFT	37.32 (36.76 to 37.88)	36.16 (34.46 to 37.86)	0.205	0.002
DSC	50.56 (49.86 to 51.26)	48.79 (46.66 to 50.92)	0.122	0.003
ln(TMT-B)	109.73 (107.23 to 112.28)	119.10 (111.05 to 127.74)	0.029	0.006
Faces	69.69 (69.20 to 70.18)	67.64 (66.15 to 69.12)	0.010	0.009
LM	27.46 (26.99 to 27.94)	27.33 (25.88 to 28.77)	0.860	<0.001
g	0.05 (−0.02 to 0.13)	−0.21 (−0.43 to 0.01)	0.028	0.006
Estimated lifetime cognitive decline				
Model 4‡				
MR	11.78 (11.45 to 12.11)	10.30 (9.24 to 11.22)	0.004	0.011
LNS	9.02 (8.83 to 9.21)	8.16 (7.60 to 8.73)	0.006	0.010
BVFT	37.11 (36.26 to 37.96)	35.95 (33.41 to 38.49)	0.394	0.001
DSC	50.61 (49.69 to 51.53)	47.22 (44.46 to 49.98)	0.023	0.007
ln(TMT-B)	109.29 (106.38 to 112.28)	124.71 (114.89 to 135.23)	0.003	0.011
Faces	69.60 (69.03 to 70.17)	67.24 (65.55 to 68.93)	0.010	0.008
LM	27.37 (26.84 to 27.90)	27.36 (25.77 to 28.95)	0.990	<0.001
g	0.06 (0.00 to 0.12)	−0.26 (−0.43 to −0.09)	0.001	0.015
Model 5§				
MR	11.81 (11.47 to 12.15)	10.28 (9.24 to 11.32)	0.007	0.010
LNS	9.03 (8.84 to 9.23)	8.24 (7.64 to 8.83)	0.013	0.008
BVFT	37.26 (36.94 to 38.13)	36.53 (33.89 to 39.17)	0.608	<0.001
DSC	50.61 (49.68 to 51.55)	48.22 (45.38 to 51.08)	0.121	0.003
ln(TMT-B)	109.18 (106.17 to 112.28)	122.73 (112.73 to 133.49)	0.010	0.009
Faces	69.70 (69.13 to 70.28)	67.32 (65.56 to 69.08)	0.012	0.008
LM	27.47 (26.93 to 28.01)	27.51 (25.86 to 29.15)	0.963	<0.001
g	0.07 (0.01 to 0.13)	−0.22 (−0.39 to −0.04)	0.003	0.012

Data are adjusted mean (95% CI). n = 807–810 for model 1; n = 806–809 for model 2; n = 775–777 for model 3; n = 798–801 for model 4; n = 767–769 for model 5. Means for ln(TMT-B) are geometric means. *Adjusted for age, sex, and baseline score. †Model 2 + baseline smoking, retinopathy, and year 4 data on HDL cholesterol, total cholesterol, systolic blood pressure, diastolic blood pressure, HbA_{1c}, stroke, TIA, angina, and MI. ‡Adjusted for age, sex, and baseline MHVS. §Model 4 + baseline smoking, retinopathy, and year 4 data on HDL cholesterol, total cholesterol, systolic blood pressure, diastolic blood pressure, HbA_{1c}, stroke, TIA, angina, and MI.

subsequent levels of cognitive function (31). However, people with type 1 diabetes typically are younger and have a lower prevalence of comorbidities than those with type 2 diabetes (32). In addition, the Diabetes Interventions and Complications Trial cohort was atypical in that the participants had been selected for having high compliance with treatment and a low risk of hypoglycemia such that these findings may have been affected by low prevalence of both the risk factor (hypoglycemia) and the outcome (age-related cognitive decline). In type 2 diabetes, one relatively small study established no links between a baseline history of severe hypoglycemia and conversion among normal cognition, impairment, and frank dementia over 18 months (14). Conversely, some evidence suggests that the experience of hypoglycemia may be a risk factor for future dementia developing in type 2 diabetes (6,7,9). Dementia lies at an end point of the continuum of age-related cognitive impairment, and to our knowledge, the current study has supplemented the current literature by providing the most robust evidence to date that exposure to severe hypoglycemia either preceding or concurrent with change in cognition during aging is associated with an increase in the rate of age-related cognitive decline in older people with type 2 diabetes without frank dementia. The finding of a relationship between hypoglycemia and the memory domain in particular is consistent with published evidence for associations of hypoglycemia with dementia, which commonly is preceded by memory impairment (33).

The findings contrast those of the ACCORD-MIND and ADVANCE trials in which patients in intensive treatment groups (with higher incidence of hypoglycemia) experienced cognitive decline at similar rates over 40 months and 5 years of follow-up, respectively, compared with the respective standard treatment groups (15,16). However, both studies were randomized controlled trials involving strict glycemic control and with cognitive function as a secondary end point. Because improving glycemic control may improve cognitive dysfunction when glycemic control is suboptimal (34), detrimental effects of hypoglycemia were potentially counteracted by the specific therapeutic interventions. The ADVANCE trial also assessed cognitive decline with a screening instrument for dementia, which is likely to be insensitive to subtle cognitive changes (35), and annual incidence of hypoglycemic episodes (defined on the basis of criteria comparable to severe hypoglycemia in the current study) was low compared with the ET2DS and ACCORD-MIND trials because none of its participants were receiving insulin treatment.

In addition to its prospective nature, the strengths of the current study lie in the relatively large size and in the population being representative of the full spectrum of people with type 2 diabetes living in the community, with treatment modalities ranging from diet to insulin, and with the inclusion of the age range at which cognitive decline often becomes apparent. A detailed battery of validated cognitive tests covered the major cognitive domains, and a reasonably comprehensive list of potential confounders was considered in the analyses, although, of course, residual confounding by any unmeasured variable cannot be ruled out. Despite potential weakness in the self-reporting of severe hypoglycemia given that not all severe episodes generate symptoms, particularly in people who have impaired awareness of hypoglycemia (36), a short prospective survey embedded into the main study demonstrated that the participants appeared to be identifying most, if not all, episodes at least of symptomatic severe hypoglycemia and that their recall (even over a number of years) was reasonably accurate. Although self-reported episodes of severe hypoglycemia may not represent all episodes of hypoglycemia, including milder episodes or symptom-free severe episodes, the measure appears to be a useful marker of a more generalized risk of exposure to hypoglycemia. One limitation of the study, which is inherent to all observational studies, is the inability to evaluate the potential for causality in the reported associations. Nonetheless, such observational studies make important contributions to the understanding of associations and inform the design of future studies aimed specifically at investigating the issue of causality. The mechanism by which hypoglycemia could potentially disrupt cognitive function is unclear. Although reports of permanent brain damage or chronic severe cognitive deficit are rare (37,38), glucose deprivation has been directly linked to neuronal death in vitro, and some evidence in type 1 diabetes suggests structural differences in the brain between patients who have and those who have not been exposed to hypoglycemia (3).

In the current study, we show that low cognitive ability is associated with an increase in the risk of subsequent episodes of severe hypoglycemia. Moreover, severe hypoglycemia at baseline and during follow-up was associated with an increased risk of subsequent and/or concurrent cognitive decline. If the latter association is found to be causal in nature, it will be necessary to address the effect of strict glycemic control on cognitive function in the clinical management of older people with type 2 diabetes. In the meantime, change in cognitive function should be considered as a clinical end point in the design of all future randomized trials of novel antidiabetes agents that have the potential to induce or augment the frequency of hypoglycemia.

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analysis. I.F. and J.F.P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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